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1. Starting point

1.1 Technological background

Microbial fuel cells (MFCs) are bioelectrical systems able to convert the energy stored in organic matter into electricity (Hernández-Fernández, et al. 2015). This process works at ambient temperature and close to a neutral pH. The principle relies on the breathing mechanism developed by certain anaerobic and microaerophilic microorganisms. The metabolic oxidation of organic compounds is a source of carbon and energy for the bacteria. Electrons are found among the final products of this metabolic oxidation, these electrons are transferred through the cell wall of the bacteria to final electron acceptors outside of the microbe. In microbes, usually oxygen is used as final electron acceptor, however, certain bacteria, called electrogenic bacteria, developed the ability to transfer their electrons (Wilson et Kim 2016) to alternative final electron acceptors to breathe with.

MFCs are made out of two compartments, an anodic and a cathodic compartment, they are separated by a proton exchange membrane (Logan, et al. 2006). Each compartment contains an electrode, respectively the anode and the cathode. The liquid phase within the anodic compartment contains soluble organic matter used as substrate by the biofilm made of electrogenic bacteria attached to the anode. The electrons released at the end of the metabolic oxidation of organic compounds by the bacteria are transferred to the anode. These electrons will flow through an external circuit to reach the second electrode, the cathode. The liquid phase within the cathodic compartment is enriched with dissolved oxygen. Oxygen acts as the final electron acceptor in the system, it is reduced into water on the cathodes surface. The electrons flowing from the anodic to cathodic compartment generate an electrical current.

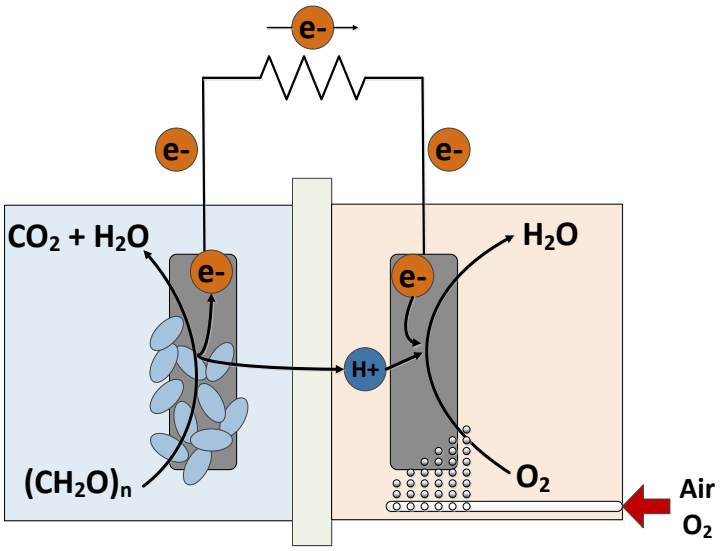

***Figure 1**: Dual chambered microbial fuel cell. The microorganisms (blue) oxidize organic matter $(CH_2O)_n$ in the anodic compartment (blue) and transfer the resulting electrons to the anode (brown). The electrons flow through the external circuit into the cathode where oxygen acts as the final electron acceptor. The anodic and cathodic compartments are separated by a proton exchange membrane (green).*

The ability of MFCs to remove organic matter while producing an electrical current makes this technology a promising approach to treat wastewater (Kishore Butti, et al. 2016). In fact, MFCs are able to fulfil the role of the aerated biological tank in wastewater treatment plants (WWTPs) by decreasing the chemical oxygen demand (COD) of wastewater influent(Pandey, et al. 2016).This alone lowers energy cost by 70%. In addition, electricity is produced at the same time and could convert WWTPs into energy producers. However, to accomplish this concept, a clear scale-up strategy should be established to overcome actual MFCs power limitations. The approach proposed by this work is to connect several MFCs in series as a stack to increase the tension of the set-up to enable intermittent power storage.

Microbial fuel cells (MFCs) are bioelectrical systems able to treat wastewater, digesting organic matter, while producing electricity.

2. Project aims

2.1 Design and construction

The first goal of the project was the design and construction of MFC stacks. The custom-made MFCs had to provide a base to study the scale-up of the technology. The scale-up strategy was to stack several MFCs in a series to increase the tension produced. In fact, like electrochemical cells, MFCs serially connected increase the potential due to the stacking (Kim, Chang et Gadd 2007) which is close to the sum of the potentials of all individual units.

Another objective was to develop an electronic system to recover and store the electricity produced by the MFC stack. Moreover, the system had to be able to monitor and manage the stack to optimize the performance of the system in real time.

2.2 Characterization of serially connected MFCs

To scale-up the technology a clear understanding of the behaviour of MFCs when serially connected is necessary. The aim was to characterize power output and internal resistances of a stack of three MFC-Units sharing a common anolyte. In fact, when MFCs are connected in series the voltage of one or several MFCs could reverse (Oh et Logan 2007) in particular with high currents. The origin of this phenomenon, known as voltage reversal, was investigated and troubleshooting methods were developed in order to balance and enhance power output.

2.3 12 Units stack MFC and an electronic energy storage system

The main objective was to set up a stack of 12 MFCs and store the electricity on lithium battery (3.7V) using an electronically controlled electricity storage system. The system had to monitor and manage the stack in real time in an autonomous manner. The idea was to control the voltage of the stack to optimize power production and recover it in a most efficient manner.

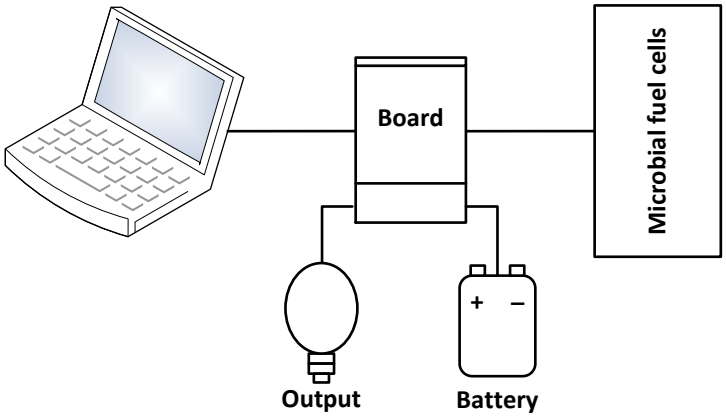

***Figure 2**: Microbial fuel cells connected to an energy storage system. The board monitored and managed the MFC-Stack, the recovered electricity was stored on a Li-battery which was then usable from an output port.*

2.4 Substrate evaluation

One of the main advantages of MFCs is their ability to convert the energy stored in organic matter present in different types of wastewaters into electrical energy. One of the goals of this project was to evaluate the efficiency of different substrates as anolyte. The properties of the different substrates in

terms of power output would determine the kind of setup in a real application as for example its use in multi-story apartment house or larger entities.

The main objectives of the project was to design and build a stack MFC. The stack was characterized and then connected to an electricity storage system. Different types of substrates were equally evaluated for potential future use.

3. Realized Work and results

3.1 Design and construction

3.1.1 Single and Stack MFC construction

Two types of MFCs were designed and built in-house in this project. The first one was a single unit MFC consisting of an anodic compartment of 500 ml facing a cathodic compartment of the same size, the two compartments were separated by a proton exchange membrane. The electrodes, anode and cathode, were made from reticulated vitreous carbon. The second reactor was a triple unit MFC based on the first construction. The anodes and cathodes shared the same anolyte and catholyte of 1.5l each. This architecture should facilitate MFC operation in waste water treatment.

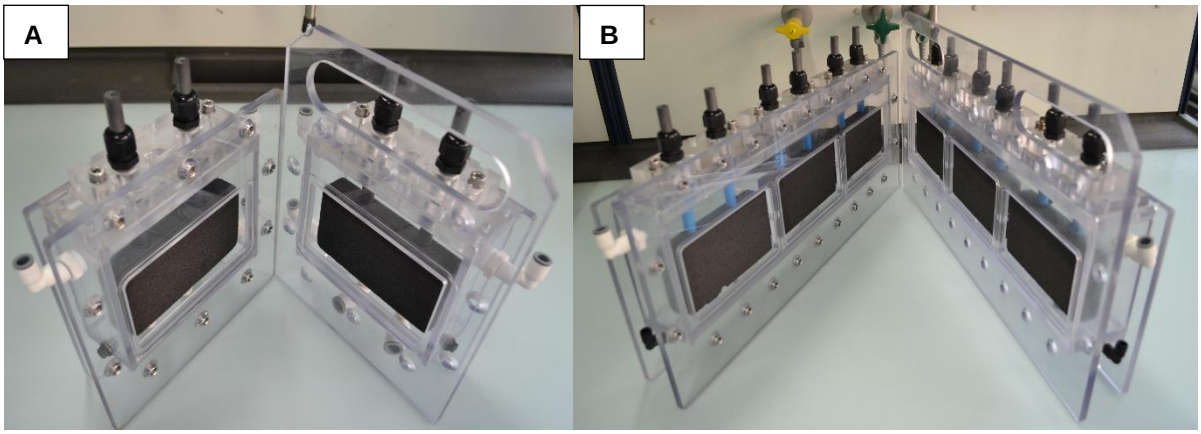

***Photo 1**: A) Two half cells of a single unit MFC, each with a capacity of 500 ml. B) Triple unit MFC, each half cell with a capacity of 1.5 l.*

A total of 5 triple units MFCs (**Photo 1**B) were built. One of them was used to characterize the behaviour of MFCs when connected in series. The four other triple MFC units were connected to an electricity storage system and run as a 12 units stack.

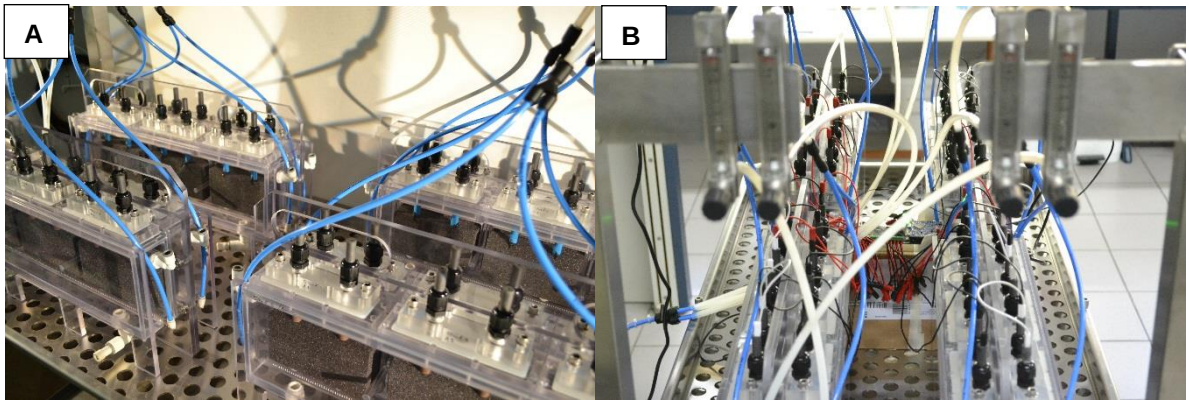

***Photo 2**: A)12 Units stack MFC able to treat 6l of waste water (anolyte).B) The stack was integrated into an energy mini-grid storage system.*

3.1.2 MFC-Stack management system

An electronic board was designed and built. The system was set up to monitor and manage 12 MFCs connected in series. The tension of each unit as well as the tension, current and power of the stack was recorded by the device. The energy recovered from the MFCs was stored by the board on a lithium polymer battery.

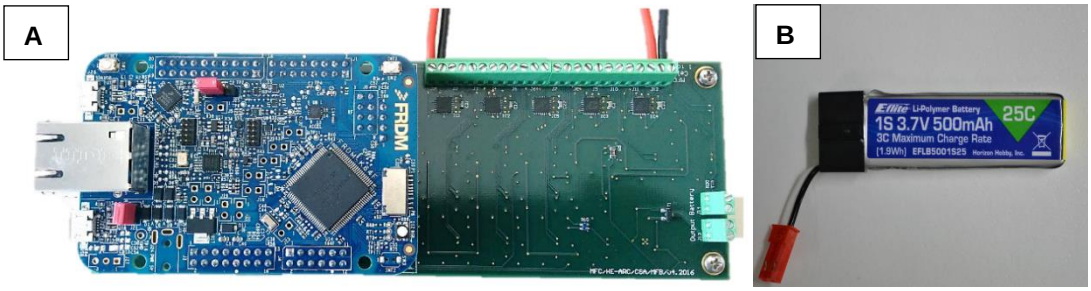

***Photo 3**: A) Electronic board to which the 12 MFC-Units were connected. It monitored and managed the stack in real time. B) Lithium polymer battery to store the electricity recovered from the MFC-Stack.*

The power management and storage device was based on the maximum power point tracking (MPPT) technique. The potential of the stack was decreased from the open circuit voltage (OCV) as long as the current increased. When the current dropped the working potential of the stack was slightly increased. The working principle of the algorithm was in perturb and observe mode, it allowed to recover as much power as possible from the stack.

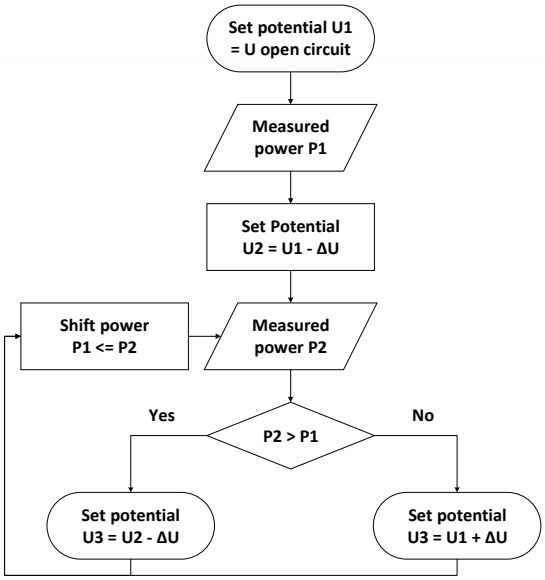

***Figure 3**: Maximum power point tracking algorithm based on the "perturb and observe" principle. The tension of the stack was adjusted in real time to optimize power production by the MFCs.*

3.2 Substrate evaluation

The real world applications of the MFC technology relies on the availability of substrates (Pandey, et al. 2016). The inherent properties of these substrates makes them more or less suitable for application and needs to be overcome. In fact, the composition and content of organic matter as well as the pH and the conductivity of the anolyte has a strong impact (Pant, et al. 2010) on the performance of a MFC.

Substrate evaluations were performed in the single unit MFC (**Photo 1** A). The conditions within the cathodic compartment were kept constant in all experiments while the anodic compartment was filled with substrates of potential interest. The biofilm on the anode was settled for an initial acclimation using primary clarifier effluent sampled at the WWTP of Châteauneuf in order to use a microbial consortia typical for municipal waste water treatment in a MFC reactor. Polarization experiments were realised in triplicate to determine the maximum power density that could be recovered for a given substrate. Also the internal resistance of the MFC was determined in this substrate characterisation experiments.

Two categories of anolytes were tested, synthetic substrates prepared in the lab and real substrates as obtained. The following synthetic substrates were prepared, characterized and tested in the MFC: simulated urine, artificial urine and artificial winery wastes. The following real substrates were tested: urine, primary clarifier effluent and cheese whey. Each substrate was characterized in addition in terms of chemical oxygen demand (COD), conductivity and pH.

3.2.1 Synthetic substrates

Synthetic substrates were newly prepared before use; their composition was defined to allow a comparison of the results. Since urine was considered in this project as a potential anolyte for future application a simulated urine was prepared and used as a standard formula. An initial assumption was that in human urine the carbon and energy source consist in 0.312 g/l, 0.048 g/l of amino acid and 0.2 g/l of proteins are also present (Ieropoulos, Greenman et Melhuish 2011). In simulated urine the carbon and energy source used was 0.312 g/l of acetate, amino acid and protein content were respectively simulated with 0.048 g/l of tryptone and 0.2 g/l of yeast extract, the compounds were dissolved in 10 mM phosphate buffer pH 7.0.

A second synthetic substrate was based on the human urine composition, it was referred as artificial urine (Rose, et al. 2015). The main difference between artificial and simulated urines relies on the fact that artificial urine composition was not only based on compounds that can be metabolised by bacteria but also on those that cannot be transformed. The composition of the artificial urine contained salts (1.18 g/l $NaHCO_3$; 4.35 g/l KCl; 6.60 g/l NaCl) and organic matter (18.2 g/l urea; 0.43 g/l uric acid; 1.93 g/l creatinine).

The third synthetic substrate, synthetic winery wastes, was based on a previous work (Sugnaux, et al. 2016) realised at the HES-SO Valais. Winery wastes are obtained at the end of the fermentation process to produce wine. One of these waste is called bourbes and is characterized by a high organic matter content (high COD) under the form of sugars (fructose and glucose). The synthetic winery waste consisted of: 85 g/l glucose; 85 g/l fructose; 0.048 g/l tryptone; 0.20 g/l yeast extract, 10 mM phosphate buffer pH 7.0.

Recorded power-current plots were normalized to the projected area of the anode and reported as power and current densities. The most efficient synthetic substrate was simulated urine with a power density max (P_{max}) of 5.0 mW/m^2 followed by the artificial urine with a P_{max} of 0.71 mW/m^2 and finally the winery waste (bourbes) with a P_{max} of 0.3 mW/m^2. Simulated urine composition was based on the metabolizable fraction of the urine, this led to a higher power density. On the other hand, the uric acid and urea present in the artificial urine are not assimilated by bacteria, the result was a lower power density. The organic matter oxidized in the anodic compartment should be compatible with the type of microorganisms present within the anode biofilm otherwise the power output of the MFC remains low and the internal resistance of the set up increases. This happened with the winery waste, the high sugar content was not compliant with the pre-existing biofilm, a specific acclimatisation is needed for such a substrate change. When substrates were not properly metabolized the internal resistance of the MFC increased. The internal resistance measured with simulated urine was 176 Ω, for artificial urine 3008 Ω and for winery wastes 1151 Ω.

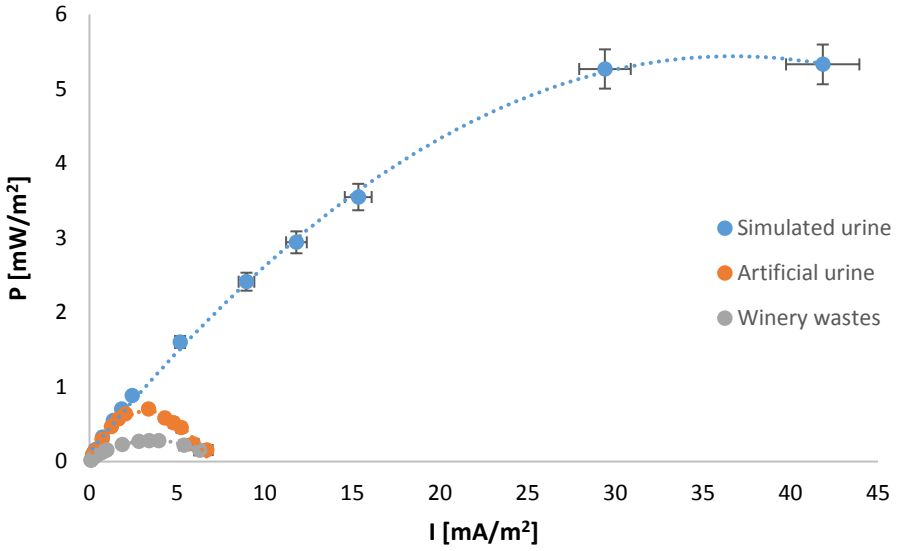

***Figure 4**: Power density curves measured in the single unit MFC with different synthetic substrates.*

The COD (chemical oxygen demand) corresponds to the amount of oxydable organic matter within a specific substrate. A high COD avoids concentration losses in the anodic compartment and supplies sufficient amounts of nutriments to the biofilm. However, the specific kind of organic matter is also of importance, the substrate should be utilizable by the microorganisms present on the anode. This was less the case for the artificial urine and the winery wastes. In fact, their COD content was higher than the COD content of simulated urine but their P_{max} values were smaller.

***Table 1**: COD, pH, conductivity and maximum power for the synthetic substrates tested in the single unit MFC.*

Substrate	COD [g/l]	pH	Conductivity [mS/cm]	P_{max} [mW/m²]
Simulated urine	0.63	7.4	1.75	5.0
Artificial urine	1.2	7.5	16.3	0.71
Winery waste	163	4.6	0.84	0.30

3.2.2 Real substrates

To achieve sustainable electricity production with a MFC, non-artificial substrates rich in organic matter should be used. Moreover, the ability of bioelectrical systems to eliminate organic matter, the main pollutant in wastewater, needs to be exploited. MFCs are thought to be, in a near future, part of wastewater treatment (Hernández-Fernández, et al. 2015) it was then necessary to test effluent withdrawn at a wastewater treatment plant. Another promising waste that was tested as anolyte in the single unit MFC was neat urine sampled from a healthy volunteer. Finally, cheese whey was tested as

substrate in the anodic compartment. In fact, due to its high organic load cheese whey treatment (Tremouli, et al. 2013) is an environmental issue in high altitude farms in Switzerland (Ticino) where milk is transformed on the spot into cheese (Project Piora, Principi 2013).

The best performance in terms of power output was achieved with neat urine with a $P_{max}$of 6.4 mW/m^2. Neat urine had a COD of 3.0 g/l and the highest conductivity tested in this project 10.8 mS/cm. Primary clarifier effluent withdrawn at the end of sedimentation tank at the wastewater treatment plant of Châteauneuf (Ville de Sion) had a lower COD and conductivity, respectively 0.26 g/l and 1.0 mS/cm. The primary clarifier effluent led to smaller power output, 2.3 mW/m^2. These two substrates are of great interest for the MFC technology. In fact, these results showed that these substrates provided a good biodegradability and are compatible with the biofilm as it is expected to be found in the anodic compartment of a waste water treatment MFC unit. Conversely, cheese whey was a substrate with a high COD content, up to 72 g/l, but the type of organic matter within this anolyte was not compatible with the bacteria on the anode. In fact, in cheese whey the organic matter content is mainly under the form of lactose (Tremouli, et al. 2013) a carbon and energy source that is not available for certain type of bacteria. A specific acclimatisation procedure that can last up to three months is needed, a time frame outside of the possibilities of this project.

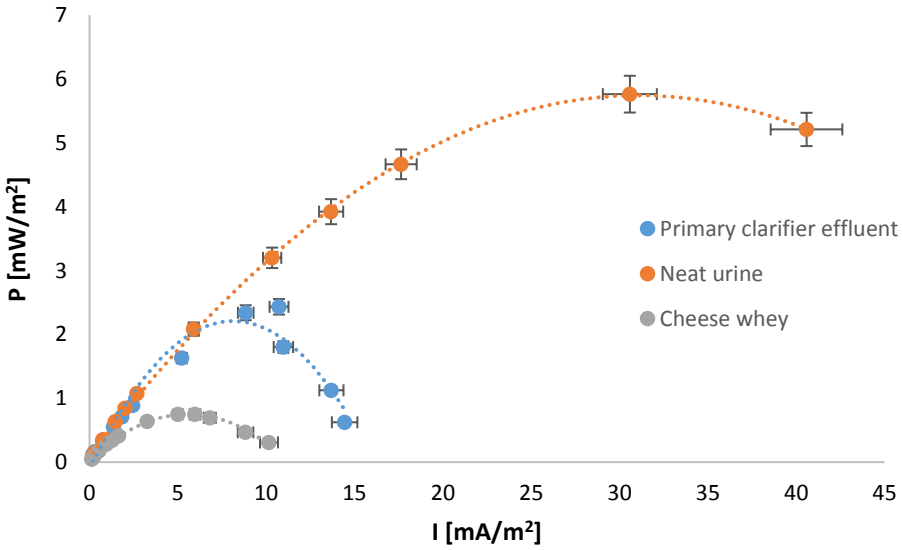

***Figure 5**: Power density curves for "industrial" substrates tested as anolyte in the single MFC-Unit.*

***Table 2**: COD, pH, conductivity and maximum power for the "industrial" substrates tested in the single unit MFC.*

Substrate	COD [g/l]	pH	Conductivity [mS/cm]	P_{max} [mW/m^2]
Primary clarifier effluent	0.26	7.9	1.0	2.3
Neat urine	3.0	7.9	11	6.4
Cheese whey	72	5.6	4.8	0.90

3.3 Characterization of serially connected MFCs

3.3.1 Triple stack MFC

The single MFC-Unit (**Photo 1A**), one anode and one cathode with a 500 ml capacity, was then scaled up to three units MFC-Stack (**Photo 1B**), consisting of three anodes and three cathodes compartments with a 1.5 l capacity each. The three MFCs sharing the same anolyte were connected in series (**Figure 6**). When several MFCs are serially connected the open circuit potential of the stack is close to the sum of the potentials of each MFC in the stack (Oh et Logan 2007).

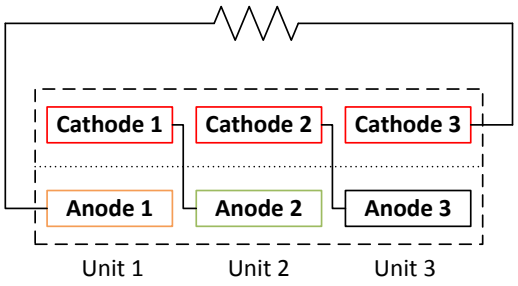

***Figure 6**: Electrical sketch of the triple unit MFC-Stack. The three MFCs (units 1, 2 and 3) were serially connected, and an external load (resistance) was placed between anode 1 and cathode 3 to close the circuit.*

The characterization of the triple unit MFC-Stack revealed the presence of a phenomenon called voltage reversal. In fact, the tension of unit 1 reversed (**Figure 7**) to become negative while the current density of the stack increased. Such voltage reversal is a sign (An, Sim et Lee 2015) of lower than possible performance of the stack. In fact, the voltage of unit 1 was subtracted from the sum of the voltages of unit 2 and 3 leading to a suboptimal power output of the stack.

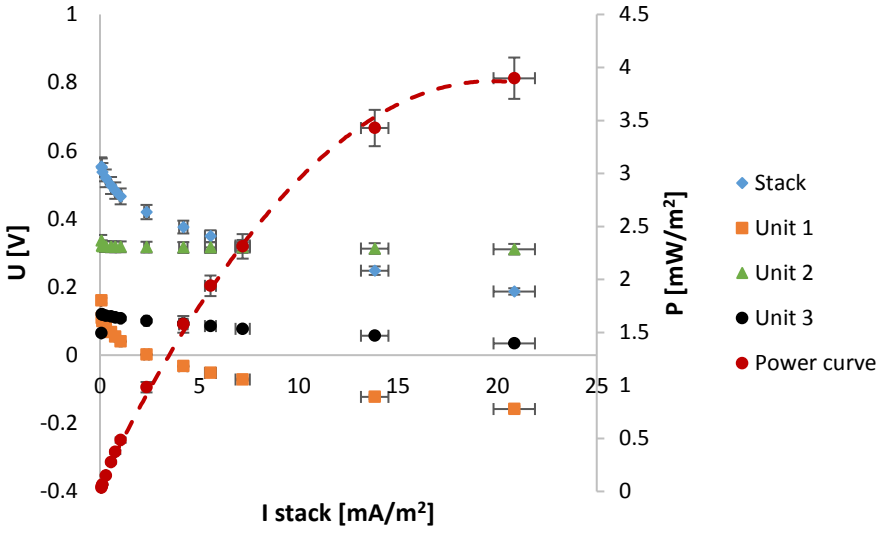

***Figure 7**: Power density and polarization curves of the triple unit MFC-Stack. Also the potential-current curves of the individual units 1, 2 and 3 of the MFC-stack. The voltage of unit 1 is reversed while the stacks current density increased.*

3.3.2 The voltage reversals origin in the serial MFC-Stack

In order to resolve the voltage reversals a clear understanding of its origin was needed to address the issue. Experiments showed that the phenomenon was related to the anodes resistance. The anode of the unit 1, the unit where voltage reversal occurred first most of the time (**Figure 7**) was permuted with the anode of the unit 3.

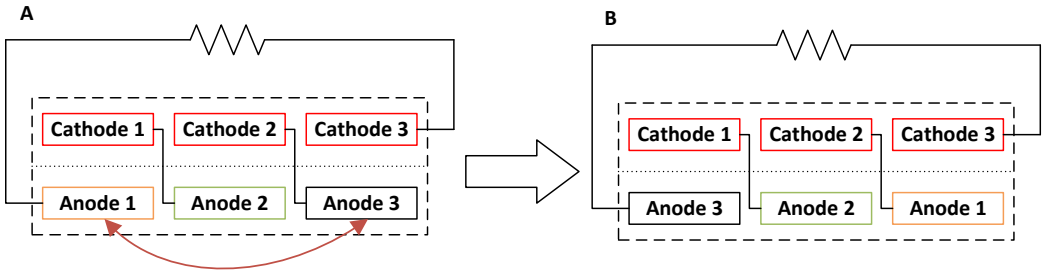

***Figure 8**: To identify the biological origins of voltage reversal the anode 1 was permuted with the anode 3. The anode 1 was part of the MFC-Unit where voltage reversal occurred, while no voltage reversal was initially present at the MFC-Unit involving the anode 3. A) Startconfiguration, B) after anode permutation.*

The voltage reversal followed the anode 1 into the new position upon permutation. Voltage reversal is a certain weakness within a MFC connected in series. In this case the critical cell component was the anode.

It was then demonstrated that the biofilm attached on the anode was the decisive factor regarding the weakness of a unit. The triple unit MFC was again characterized once it was balanced, meaning that

no voltage reversal occurred when the stack current reached highest values. The anode 1 was then replaced by an anode with the exact same properties except that the anode was free of biofilm.

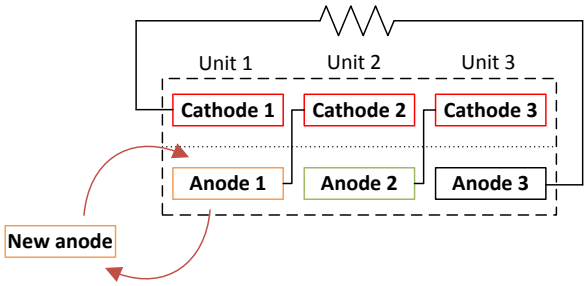

***Figure 9**: The anode 1 colonized by a biofilm was replaced by an anode free of biofilm. The triple unit stack MFC was characterized before and after the replacement.*

Again the biofilm was of primary importance to ensure proper operation of a cell within a stack. In fact once the anode 1 was replaced with a biofilm free anode a very strong voltage reversal was observed at unit 1.

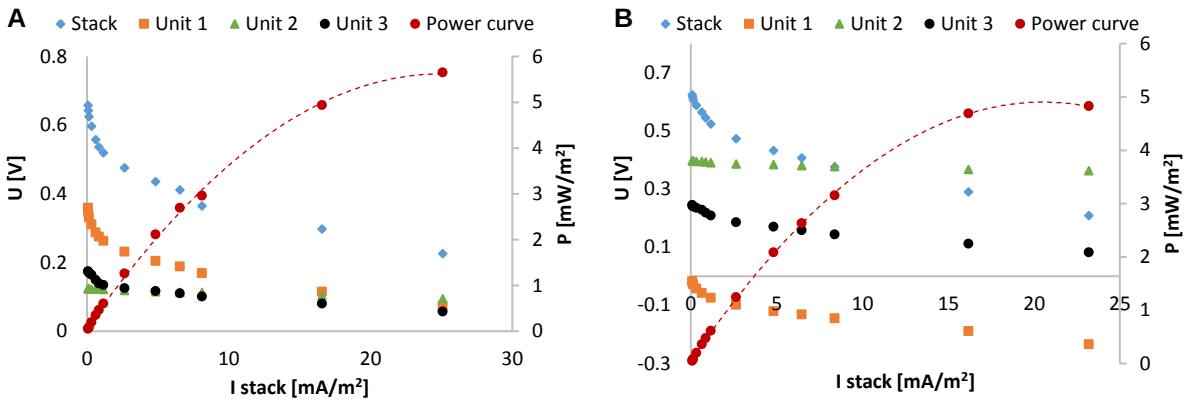

***Figure 10**: A) The triple MFC-stack in a well balanced state at higher current densities, no voltage reversal was detectable. B) The same stack where anode 1 was replaced by a biofilm free anode, the voltage of unit 1 reversed even at low current densities.*

3.3.3 Voltage reversal and internal resistance

At this point the relation between the anode, more specifically the biofilm, and voltage reversal was established. The direct consequence of a weak biofilm was the relatively higher internal resistance of the MFC-Unit. It was observed that the cell with the highest internal resistance in the stack was the cell where voltage reversal occurred, and this can occur even in a relatively well performing stack.

The relation between internal resistance and voltage reversal was first demonstrated with a non-biotic cell designed and tested. The triple unit MFC-Stack was simulated, each unit was set-up with different internal resistances respectively 1000 Ω, 560 Ω and 470 Ω.

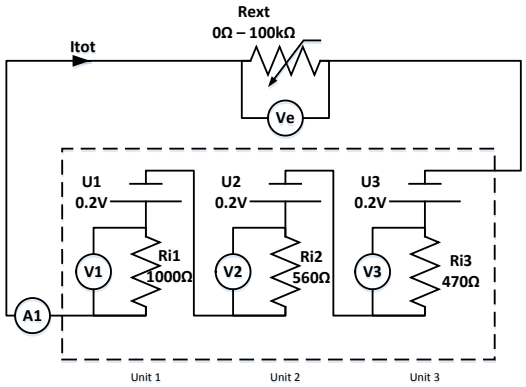

***Figure 11**: Non biotic cell designed to mimic the triple unit MFC-Stack. Each unit had a different internal resistance respectively 1000 Ω, 560 Ω and 570 Ω.*

A polarization experiment was run with this non-biotic stack. While the current increased the voltage of unit 1, the unit with the highest internal resistance, reversed first. No voltage reversal was observed for unit 2 and 3.

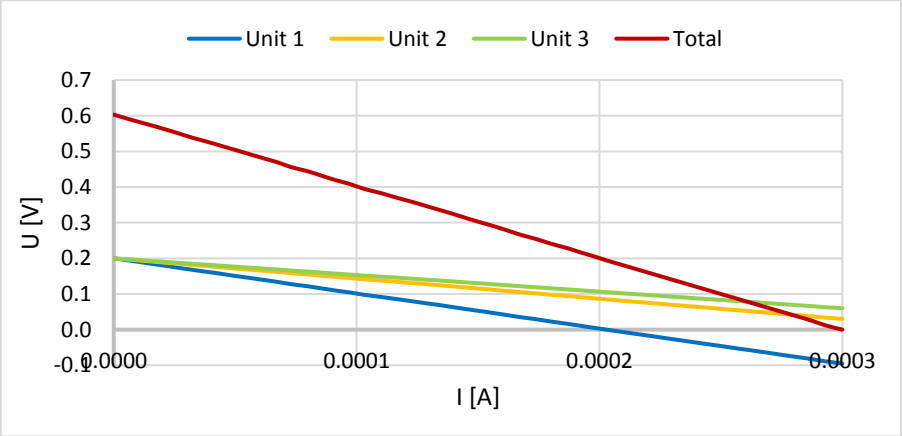

***Figure 12**: Polarization experiment with the non-biotic stack. The voltage of unit 1 reversed while the current increased. Unit 1 was the unit with the highest internal resistance.*

This experiment demonstrated the relation between voltage reversal and internal resistance. The unit with the highest internal resistance within a stack will see his voltage reversed first while the current increases.

3.3.4 Voltage reversal troubleshooting in serially connected MFC-Stacks

Five solutions were proposed and tested to eliminate voltage reversal in serially connected MFCs, namely:

- Nutrient increase
- Recirculation
- Circuit alternation

- Anode permutation
- Electrostimulation

Concentration losses occur when the mass transfer rate of substrate to the biofilm becomes the limiting factor, usually observed at high current density it could lead to voltage reversal. The first solution to overcome this problem, nutrient increase, consisted in increasing the substrate concentration within the anolyte to improve mass transfer from the bulk to the anodic biofilm. However, increasing the substrate concentration by a factor 5 did not solve the voltage reversal. The second solution proposed relied on the same principle, recirculating the anolyte postponed voltage reversal, this was achieved by adding a peristaltic pump to the set-up. The recirculation of the anolyte at a flow rate of 3 l/h restored the performance of the stack overnight by eliminating the light voltage reversal initially located at unit 3 (**Figure 13**). The maximum power density was increased from 6.1 mW/m^2 up to 8 mW/m^2.

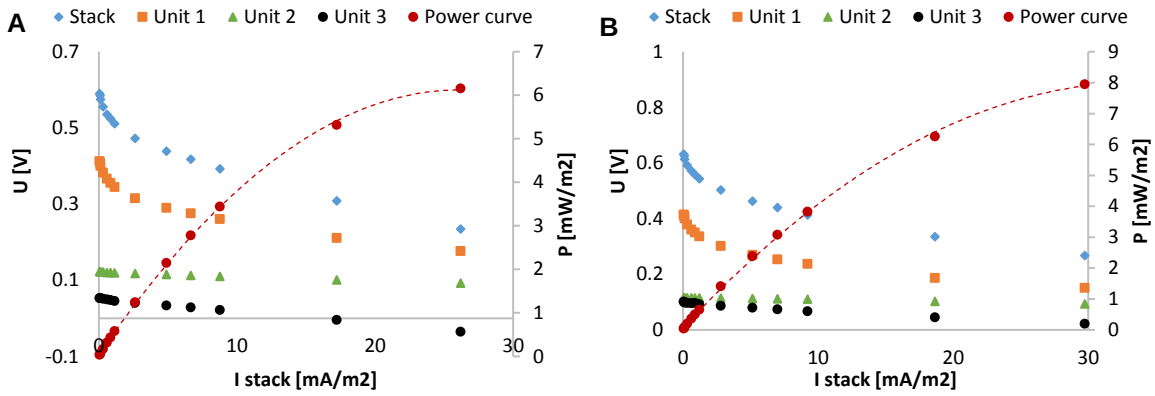

***Figure 13**: A weak anode showed a minor voltage reversal initially at unit 3.In the beginning the anolyte was not recirculated by the branched peristaltic pump. B) The stack was balanced and voltage reversal eliminated once the anolyte was recirculated at a flow rate of 3 l/h.*

The third solution, circuit alternation, consisted in a reconfiguration of the electrical circuit. In fact, it was observed that the location of the external resistance was linked to the location of voltage reversal. An external resistance needs to be added to close the circuit and shows what happens if there is a load that uses produced electricity. The anode connected to the external resistance was disadvantaged over the other anodes. In fact, the presence of the external resistance was an obstacle for the electron flow. In MFCs electron transfer to the anode is the breathing mechanism adopted by the biofilm, it is vital for the bacteria. The biofilm on the anode connected to the external resistance was weakened over long time operation due to the decrease of the electron flow. As previously explained in section 3.3.2 a weak biofilm would lead to voltage reversal. To avoid the settlement of disadvantaged biofilms the external resistance was displaced from one extremity of the stack to the other.

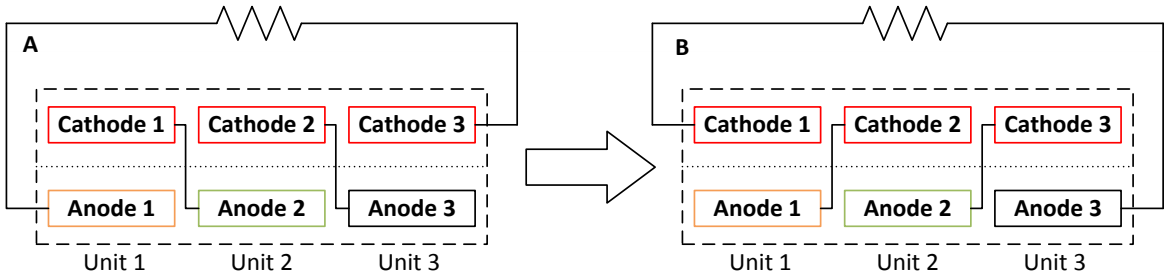

***Figure 14**: A) The external resistance (load) was initially located between anode 1 and cathode 3. Voltage reversal settled at unit 1, the anode 1 was disadvantaged over the other anodes in terms of electron flow. B) The external resistance was displaced at the other extremity of the stack to stimulate anode 1 recovery.*

At the beginning of the experiment voltage reversal was located at unit 1 (**Figure 15** A). The anode 1 was connected to the external resistance (**Figure 14** A) and then disadvantaged over the anodes 2 and 3. The external resistance was displaced on the other side of the stack (**Figure 14** B) and the reactor was run under the new configuration to allow the anode 1 to recover. After 10 days of operation the stack was balanced, voltage reversal was eliminated and the power density increased from 3.8 mW/m^2 up to 5.4 mW/m^2 (**Figure 15** B).

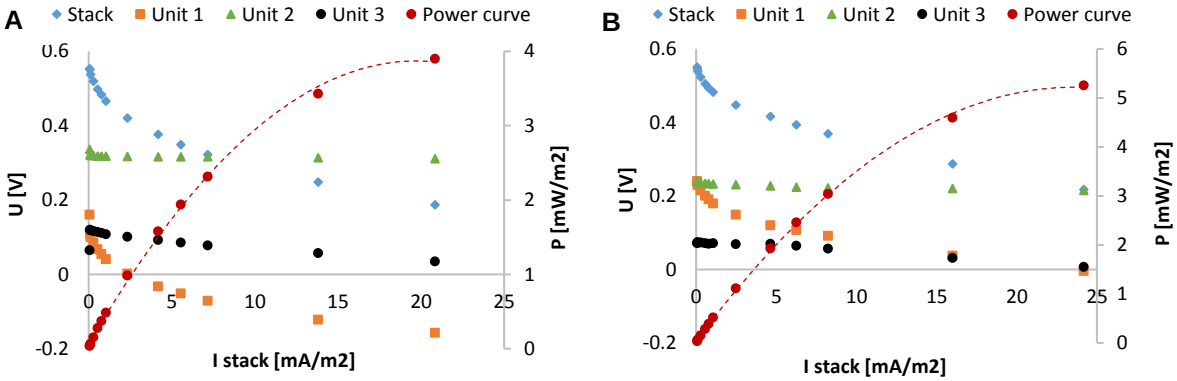

***Figure 15**: A) The external resistance was located between anode 1 and cathode 3, voltage reversal settled at unit 1. B) The circuit was alternated, the external resistance was then located between anode 3 and cathode 1. After 10 days of operation voltage reversal at unit 1 was eliminated and the stack was in a more balanced state.*

The permutation of a weak anode with a healthy anode (**Figure 8**) could enhance the vitality of a weak biofilm. This technique relies on the same principle than circuit alternation (**Figure 14**), when the electron flow of a weakened anode is increased due to his location within the stack, vitality of the biofilm could be recovered.

Finally, applying a potential to stimulate a weak anode was the fifth solution to solve the voltage reversal. A power supply was used to apply 0.4 V to a weak anode to restore the performance of the cell and then eliminate voltage reversal. When a potential was applied to the unit, the anode acted as an electron sink, the electron transfer rate from the biofilm to the anode was greatly enhanced. Voltage reversal was eliminated over 2 days with this technique. The stack was balanced and the maximum power density increased from 2.9 mW/m^2 up to 7.3 mW/m^2.

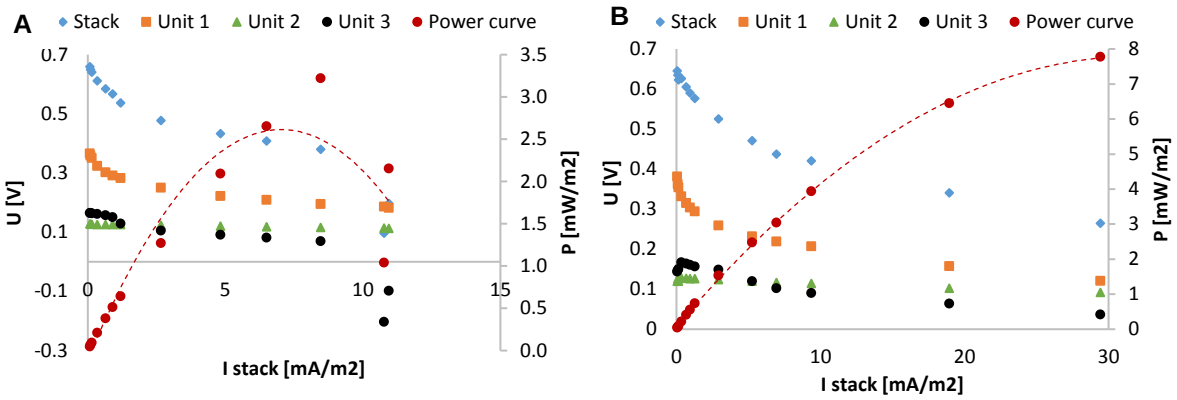

***Figure 16**: A) Voltage reversal was initially located at unit 3. B) 0.4 V was applied to unit 3 overnight for 2 days, and voltage reversal was eliminated.*

3.4 12 Units MFC-Stack

3.4.1 Three week run (milestone)

The integrated use of a stack of 12 MFCs serially connected with an electronic electricity storage system was a key step in this project. Four triple units MFC-Stacks were assembled (**Photo 2**). Finally a total of 12 MFCs were serially connected through the aforementioned electronic board (**Photo 3** A), which was used to monitor and manage the stack. The energy recovered was stored on a lithium polymer battery of 3.7V (**Photo 3** B).The stack was run over three weeks under a fed batch regime. Each week, both anolyte and catholyte were renewed, the battery was replaced on a weekly basis.

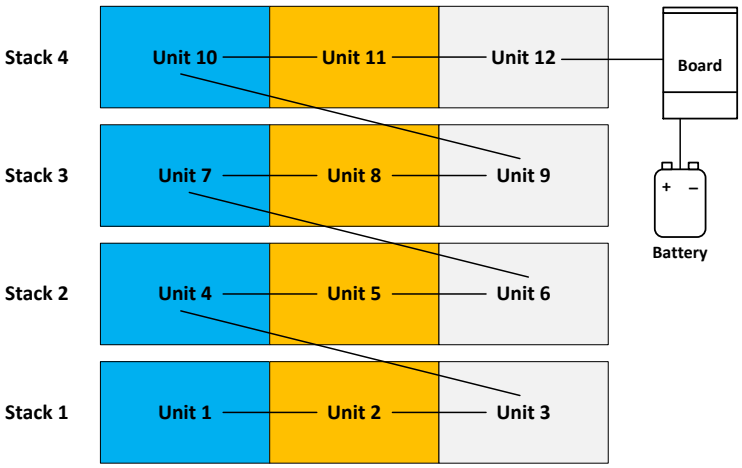

***Figure 17**: Four triple units MFC-Stack (stacks 1, 2, 3 and 4) for a total of 12 MFCs (Units 1-12) were serially connected, managed and monitored by an electronic board. The recovered electricity was stored on a lithium polymer battery.*

The stack was managed according to the maximum power point tracking technique (**Figure 3**). The working potential of the stack was automatically adjusted by the board (**Photo 3** B) to recover as much power as possible. The potential was decreased from the open circuit potential (no current, infinite

resistance) as long as the power increased. The open circuit voltage of the stack was 2.1 V, it was decreased by the board over the first days of operation to 0.82 V. Maximum power was recovered near this voltage and a peak of 1.19 mW was measured in the first week of operation. Once the substrate started to be depleted the power decreased until the anolyte was renewed.

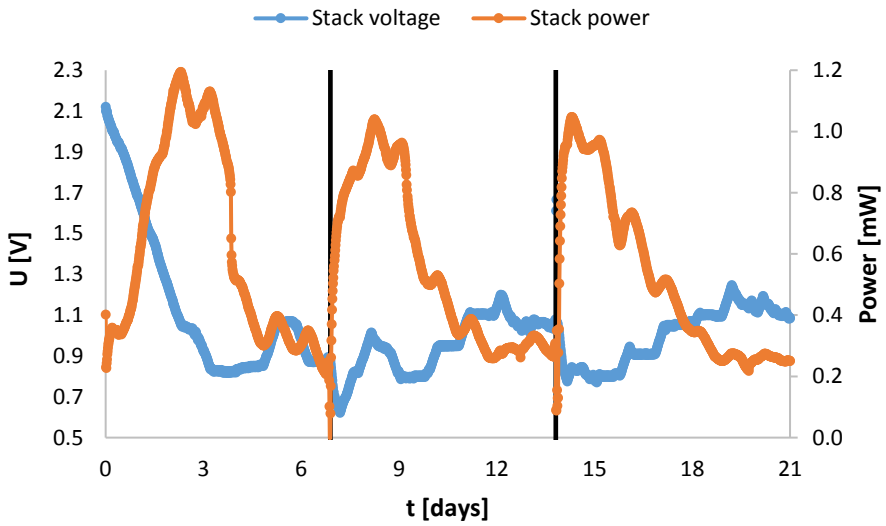

***Figure 18**: Potential and power of the 12 MFC-Stack serially connected and managed in real-time by the electronic board. Vertical bars (black) represent weekly anolyte and catholyte renewal, the MFC-Stack was run under a fed-batch regime.*

The electricity recovered from the serially connected MFC-Stack was stored on lithium polymer batteries having a capacity of 500 mAh for a nominal voltage of 3.7 V. Three batteries were successfully charged over the three weeks of experiment from an average tension of 2.55 V up to an average tension of 3.55 V (**Figure 19**).

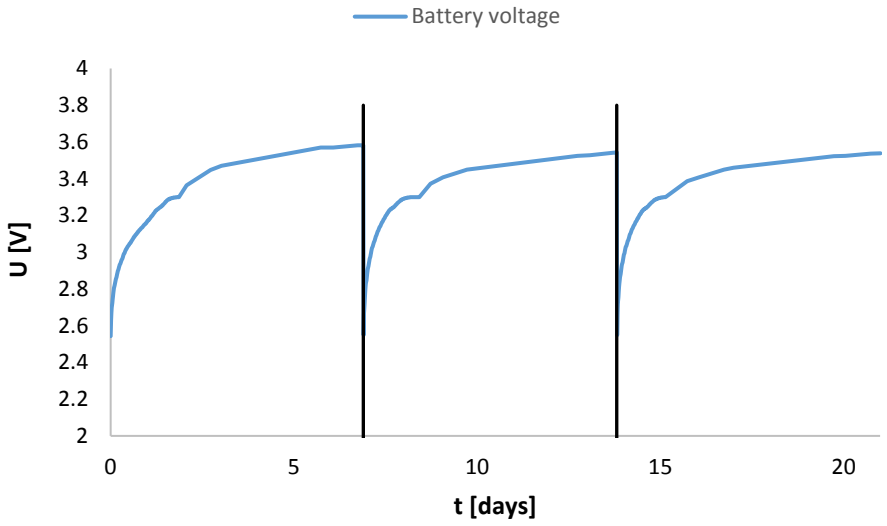

***Figure 19**: Charging degree controlled by an increasing potential of the batteries loaded by the serial 12 MFC-Stack. Vertical bars indicate anolyte and catholyte renewal as well as battery replacement points.*

3.4.2 Simultaneous loading of 3 batteries

To optimize the energy recovery from the 12 MFCs the stack was assembled in alternative configurations and split here into 3 stacks (A, B and C) of 4 units. Each stack was monitored and managed by its own power management board in an in depend manner. With this set-up three batteries were charged simultaneously. Under this configuration the serially connected MFC-Units did not share a common anolyte, what increased the working potential in comparison to the one dimensional setup that was described before.

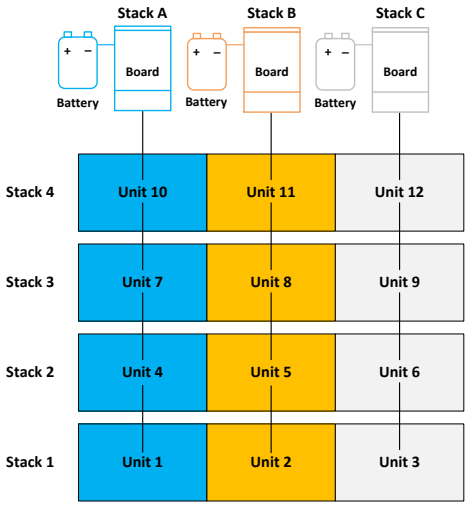

***Figure 20**: 12 Units MFC-Stack run in a sort of 2D configuration where three stacks were made with four MFC-triple type Units each. The three charging stacks were managed and monitored by three independently working electronic control boards.*

Each board started to decrease the working potential of the stacks A, B and C starting from the OCV. The working potentials were decreased as long as the power increased according to the maximum power point tracking technique (**Figure 3**).

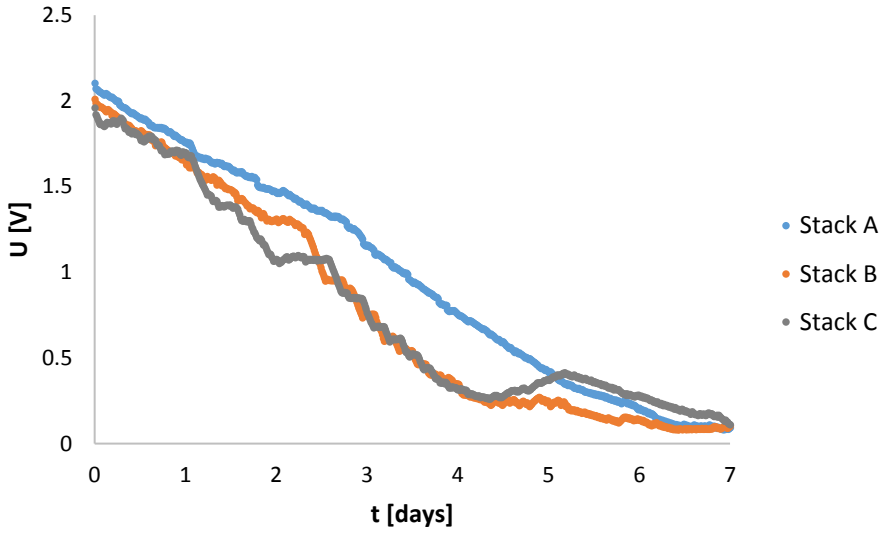

***Figure 21**: Charging potentials of the stacks A, B and C controlled by their respective board over 7 days. The 12 MFCs were run as 3 stacks of 4 units, the units within each stack did not share a common anolyte in order to increase the working potential with less units, what should be beneficial when unit potentials drop.*

The charging potentials of stacks A, B and C were slightly different. In fact, the potential of stack C remained, for most of the experiment below the values of the stacks A and B. In the middle of the experiment the potential of stacks B and C were matched closely, in an oscillating manner (**Figure 21**). A lower potential resulted in an increase of the current for the concerned stack and an increase of the power recovered. It was observed that the stack with the lowest working potential drained most of the power over the whole experiment. In this case, stack C was favoured over stacks A and B. The oscillation between the tensions of stacks B and C is also present on the power curves (**Figure 22**) measured over time.

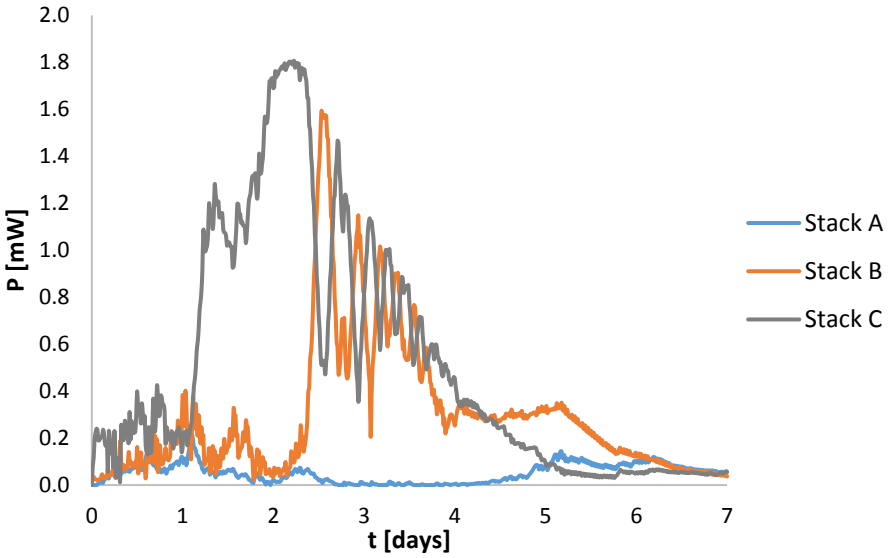

***Figure 22**: Power curves over time from stacks A, B and C. The 12 MFCs were run as 3 stacks of 4 MFCs with three units that did not share the same anolyte. Most of the power was recovered from stack C, the stack with the lowest charging potential.*

Here three batteries were successfully charged simultaneously. The charge of batteries B and C were comparable to the charge of the battery in the previous experiment in which a single battery was charged with the entire stack (12 MFCs) during the same time. In fact battery B was charged up to 3.4 V, battery C was charged up to 3.5 V with their respective stacks of 4 serially connected MFCs that did not share a common anolyte. In previous experiment, the battery was charged up to 3.55 V by 12 serially connected MFCs with common anolyte. The charge of the batteries was proportional to the power recovered over the experiment (**Figure 22**), the charge of battery A was then lower than other stacks B and C.

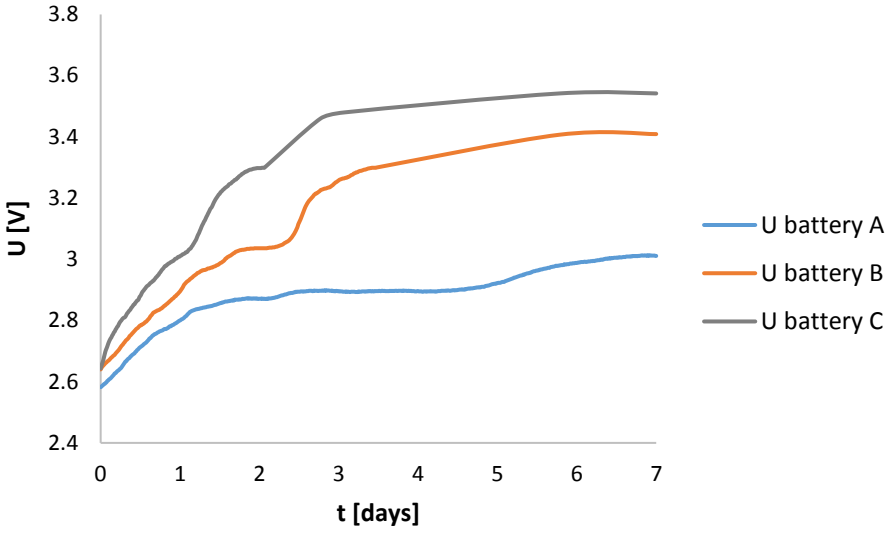

***Figure 23**: Tensions of the batteries A, B and C charged by their respective stacks of 4 serially connected MFCs without shared anolyte.*

As in previous experiments other parameters not directly related to electricity were examined. Chemical oxygen demand (COD) was assayed at the beginning and the end of the process. One of the aim of MFCs is to decrease, by microbial digestion, the organic matter concentration (COD concentration) in wastewater effluent. In this particular experiment, 3.37 g of COD were initially present within the 6 L of anolyte treated in the stacks 1, 2, 3 and 4. After 7 days of experiment, 0.78 g of COD were still present within the anolytes, 76.7 % of the oxydable organic matter present at the beginning of the experiment was eliminated (final COD concentration 131 mg/l). The fraction of COD consumed increased up to 89.7 % when the experiment was repeated for a duration of 14 days (final COD concentration 64 mg/l). According to the waters protection ordinance 814.201 of the Swiss Confederation a removal efficiency of 85 % is required for wastewater treatment plants treating wastewater for more than 10’000 people. The removal efficiency requirements were achieved with the MFC-Stack when run for 14 days (89.7 % removal efficiency).

Three set-ups were designed and built, a single unit MFC, a triple unit MFC and a stack of 12 MFCs. The characterization of the triple unit MFC revealed the presence of a phenomenon called voltage reversal, this phenomenon had a negative impact on the performance. The origin of voltage reversal was identified, five measures were developed and tested to restore the performance of the MFCs.

4. Business plan

4.1 Determining the boundary conditions

A rough business plan was realized by our project partner Sibylle Duttwiler (Duttwiler Energietechnik). The goal was to find out whether the technology microbial fuel cell would suit the targeted application, and under what conditions. Therefore, a calculation was executed that showed:

- a minimal efficiency needed of 70W/m^2 for the Electrode (Anode or Cathode)

If surface efficiency was lower, to much surface has to be manufactured and built-in the unit and the unit would become to expensive.

- a minimal substrate energy content needed of 50gCOD/L

more diluted or less energy intense substrate would not generate the targeted power output and therefore don't fit into the business plan. So substrates like slurries, diluted faeces are not applicable for that business case. Substrates like whey (sweet or sour) or undiluted dung must be the targeted.

To find a market gap it was assumed to need

- a minimal device size of 350 W, scalable to 1000 W

(under this 350 W solar cells are assumed to be to strong competitors, whereas for larger size other systems like biogas are assumed to fit more).

Under these boundary conditions (irrespective whether they are at the moment reachable or not), the following business plan calculations were conducted. Furthermore, material prices where estimated as future prices, less expensive than they are today. On the other hand, it was calculated with Swiss wages and with small batch series manufacturing.

4.2 Strategy

The strategy proposed was to sell or rent (for seasonal operation) two different models of ready-to-use MFCs reactors able to produce 350W and 1000W, respectively. The installation as well as the maintenance would be ensured by the company. Another possible product would be “starter kits”, the starter kits contain the nutrients and bacteria necessary to the MFCs acclimation (a product, that would have to be developed in cooperation with the university Sion).

MFCs offer to exploit the energy potential of organic waste while simultaneously solving the disposal problem that such wastes represent. The target markets are customers who combine the requirements of waste disposal and on site clean energy production. Potential customers are:

- Cheese factories and milk processing plants
- Restoration companies
- Mountain hut
- Allotment
- Mobil toilet systems

At the beginning the geographical market would be limited to Switzerland since the system initially requires installation and maintenance.

The main competitors would be companies who offer small to medium scale power production plants. Manufacturers of methanol fuel cells, photovoltaic solar cells or small wind turbine are the main examples. To clearly state the strengths and weaknesses of the business plan a SWOT analysis was realised.

***Table 3**: SWOT analysis of the microbial fuel cell stack.*

Strengths • It produces electricity and helps to save energy. • The energy recovered from the oxidation of the organic matter can be stored on batteries. • The project demonstrated that the scale-up of such technology is feasible by connecting several MFCs in series. • MFCs stacks are compatible with current electrical management systems. MFCs could then be integrated into pre-existing electrical grids.	**Weaknesses** • The electrodes are rather expensive • Proton exchange membranes are critical components that would need to be periodically replaced. • Current and power densities are still low. To build a reactor that can produce 350W (see section 4.1) about 23'000 m^2 of anode would be required. • Solving voltage reversal can take several days.
Opportunities • Double benefit: waste disposal and energy production. • Applicable with a wide range of substrate. • Wastes are locally treated; transport is no longer required. • Helpful to comply with more and more restrictive environmental laws. • Reduces waste water treatment electricity consumption by 70%.	**Threats** • The materials used to build the reactors are expensive and won't become cheaper. • Technical know-how can be developed elsewhere. • Product not accepted by the target market.

4.3 Economic objectives

As previously stated different sources of revenue are needed:

- Sales of 350Watt-unit
- Sales of 1000Watt-unit
- Income from maintenance work
- Government (or other) support for evolving environmental technologies
- Sales of Starter/Care kits
- Income from rentals of 1000Watt-units.

A detailed presentation of the economic objectives over four years regarding the sales of the 350W-reactor is presented in this report (**Table 4**) to illustrate the reasoning of the economic analysis. Same economic analysis were realised for the other sources of income (available in a separate report), a summary is presented in **Table 5**.

***Table 4**: Economic objectives regarding the sale of 350Watt- units over the first four years of exploitation.*

	2018	**2019**	**2020**	**2021**
Amounts [units]	15	20	40	60
Price [CHF/unit]	6000	5500	5000	4500
Material [CHF/unit]	3200	2700	2200	2200
Contribution margin [CHF/unit]	2800	2800	2800	2300
Gross sales [CHF 1,000]	90	110	200	270
Net sales [CHF 1,000]	90	110	200	270
Gross profit [% sales]	47	51	56	51
Growth [% net sales]	0	22	82	35
Total contribution margin	42000	56000	112000	138000

Table 5: Summary of the number of units and net sales over four years for the different sources of income.

	2018		2019		2020		2021	
	Amounts [units]	Net sales [CHF 1,000]	Amounts [units]	Net sales [CHF 1,000]	Amounts [units]	Net sales [CHF 1,000]	Amounts [units]	Net sales [CHF 1,000]
Sales 350W-unit	15	90	20	110	40	200	60	270
Sale 1000W-unit	5	65	10	120	16	160	28	252
Maintenance work	8	14	10	18	20	36	30	54
Environment funding	1	30	1	30	1	30	1	30
Sales starter/care kits	0	0	0	0	100	9	300	25
Rental 1000W-unit	2	10	4	19	6	27	8	36
Net sales tot.		**209**		**297**		**462**		**667**

4.4 Expenses

Five sources of expenses were identified:

- Marketing
- Project innovation
- Staff
- Infrastructure
- Operating expenses

To reach customers, marketing measures would be important. A website, providing information about the company and the product would be set-up, publications as well as small-scale material (flyers) would also be provided. Potential customers would by directly asked for consultation and open days will be organized to present the reactors. The second source of expenses, project innovation, is mainly based on research to optimize the material cost and efficiency and develop starter/care kits. The infrastructure and operating expenses are basic requirements for all type of companies, vehicle and transport costs, insurance, energy and waste disposal are typical examples. Regarding the staff, at the beginning, the different functions will be assumed by 2 people, after four years 5 people would be necessary according to the proposed business plan. A detailed economic analysis of each expense source is available in a separate report, the costs are summarized in the following **Table 6**.

Table 6: Summary of the expenses expected over four years of exploitation. The amounts are given in CHF 1,000.

	2018	2019	2020	2021
Marketing	45	18	23	17
Project innovation	5	4	2	20
Staff	66	98	176	238
Infrastructure	14	18	28	31
Operation	9	14	21	27
Costs tot.	**139**	**152**	**250**	**333**

According to the economic analysis (**Table 5**, **Table 6**) profit would be made from the second year of exploitation. The cost of the material to build the reactors are taken into account, the number of units for each year are given in **Table 5**.

Table 7: Profit before interest and taxes, the values are given in CHF 1,000.

	2018	2019	2020	2021
Income (**Table 5**)	209	297	462	667
Material	-89	-109	-177	-288
Expenses (**Table 6**)	-139	-152	-250	-333
Profit	**-19**	**36**	**35**	**46**

The business plan was based on a reactor able to reach a power density of 70 W/m^2 for a total of 350W. The strategy was to sale or rent MFCs to facilities that required on site waste treatment and that could benefit from the energy production of the system (cheese factories and milk processing plants, restoration companies, and mountain hut).

5. Conclusions

5.1 Characterization of serially connected MFCs

An initially constructed single unit MFC was scaled-up to a triple unit MFC (**Photo 1** B), the initial 500 ml anolyte capacity increased to 1500 ml. The three units were serially connected, the open circuit potential reached by the stack was the sum of the potentials of the three units of the stack. A phenomenon called voltage reversal was identified during the characterization of the stack. In fact, while the current of the stack increased the tension of one unit reversed to become negative impacting the performance of the stack. This phenomenon was symptomatic for an unbalanced stack. It was

established that voltage reversal was linked to the anodic biofilm. A demonstration for this assumption was realised by permuting the anode of a weak unit with the anode of a healthy unit (**Figure 8**), voltage reversal was located at the unit involving the weak anode. Another proof was realized by inserting an anode free of biofilm (**Figure 9**) in the stack, the voltage of the unit with the new anode became negative. It was then demonstrated that voltage imbalance was the manifestation of internal resistance imbalance. A non-biotic cell was designed (**Figure 11**) to simulate three MFCs serially connected, each unit was set up with a different internal resistance, respectively 1000Ω, 560Ω and 470Ω. While the current was increased the tension of the unit with the highest internal resistance reversed (**Figure 12**). In conclusion, a weak anodic biofilm will as a consequence increase the internal resistance of the concerned cell. An imbalance between the respective internal resistances of the cells will result in voltage reversal of the cell with the highest internal resistance at high current density.

The measures to solve voltage reversals (see section 3.3.4) were based on two principles, increasing the mass transfer rate of the substrate from the anolyte to the biofilm and stimulate the electron transfer rate from the biofilm to the anode. Both principles had the same goal, restore the vitality of a weakened biofilm to balance the stack. To limit concentration losses, the concentration of the substrate was increased by a factor of 5. Experimentation revealed that this technique was not the most efficient to balance the units. The second solution, anolyte recirculation, postponed voltage reversal (**Figure 13**) by recirculating the anolyte at a flow rate of 3 L/h. This result is promising since in future applications MFCs will be operated in continuous mode. The third solution remained on the observation that the anode connected to the external resistance close the circuit was disadvantaged over the others anodes. In fact, the electron flow coming from this anode was limited by the presence of the external resistance that simulated any load in an application. Alternating the circuit (**Figure 14**) by external resistance displacement from one extremity of the stack to the other effectively balanced the stack (**Figure 15**) in 10 days. An alternative to circuit alternation would be anode displacement. Voltage reversal could be eliminated by inserting a weak anode in a more favourable environment to regain proper biofilm activity. Finally, the electrostimulation was the fastest method and a good solution to eliminate voltage reversals. A tension of 0.4V was applied overnight to a weak unit to stimulate the electron flow. After 2 days of electrostimulation (**Figure 16**) the stack was properly balanced. In conclusion, mass transfer limitation within the anodic compartment should not be a problem for future concrete applications if a continuous regime implying anolyte circulation is chosen. MFCs can be balanced with soft measures like circuit alternation or anode permutation, these techniques do not require the input of external sources of energy. Finally, electrostimulation offers a rapid alternative to restore the activity of a weak anode but it requires an external power supply.

5.2 12 Units MFC-Stack

Four triple unit MFC-Stacks were assembled (**Photo 2**) to carry out the scale-up strategy. An electronic board was designed and built (**Photo 3** A) to monitor and manage the 12 units MFC-Stack.

The electrical energy recovered was stored on lithium polymer batteries (**Photo 3** B). The 12 units were serially connected (**Figure 17**) and run over three weeks in fed batch mode, both anolyte and catholyte were renewed on a weekly basis. Three batteries were successfully charged (**Figure 19**), one per week, performing the experiment. The stack was managed according to the maximum power point tracking technique (**Figure 3**) to optimize, in real-time, the power production. The tension of the stack was decreased as long as the power increased, peaks of power up to 1.2 mW were obtained (**Figure 18**). The requirements regarding the management system were fulfilled, the maximum power point tracking technique was compatible with the MFC-stack operation. The optimization of power production in real time is an advantage for MFCs, a living system in which slight variations could be encountered during the operation. Finally, the scale-up strategy that consisted in serial connexion of several MFCs to treat higher anolyte volumes allowed to reach OCV value as high as 2.1 V.

The 12 units were reorganised into 3 stacks of 4 units that did not share a common anolyte (**Figure 20**). The stacks were managed by their own board independently of each other. This two dimensional configuration allowed to simultaneously charge three lithium batteries. The charge of two of them was of the same importance (**Figure 23**) than in the previous experiment realised with 12 units serially connected (**Figure 17**). In fact, the working potential of the cells was improved when compared to the working potential obtained with the one dimensional configuration. This set-up is of great interest since it offered the possibility to load three batteries with 12 cells while a single one was loaded with the one dimensional configuration.

5.3 Substrate evaluation

The single unit MFC (**Photo 1** A) fulfilled its role as a support to investigate the efficiency of different substrates. Among the synthetic substrates tested (see section 3.2.1) the most efficient was the simulated urine, a substrate based on the fraction of human urine that can be metabolised by bacteria. In fact, the P_{max} reached with simulated urine (5.0 mW/m^2) was 7 times higher than the P_{max} reached with artificial urine (0.71 mW/m^2), a substrate that included not only the metabolically transformable compounds but also compounds that cannot be transformed by bacteria. This first set of data revealed the importance of the compatibility of the substrate with the metabolism of the biofilm. The third synthetic substrate tested confirmed this observation. In fact, artificial winery wastes contained the highest amounts of organic matter (**Table 1**) when compared with simulated and artificial urines but led to the lowest performance in terms of maximum power output (0.30 mW/m^2) due to a lack of specific microbes, which need to be sourced. Among the real substrates tested (see section 3.2.2), cheese whey was inefficient here while urine and primary clarifier effluent showed promising results. As previously observed with synthetic substrate the biofilm could not assimilate every types of substrate, it was the case for cheese whey (P_{max} 0.90 mW/m^2). Cheese whey is mainly composed of lactose a non-compatible organic compound for the present anodic biofilm. A $P_{max}$of 2.3 mW/m^2 was achieved with primary clarifier effluent despite the low COD content (**Table 2**) of this substrate. This

result confirmed that primary clarifier effluent could easily be treated by MFCs and support the concept of integrating this technology in wastewater treatment plants. The highest P_{max} was achieved with urine as anolyte (6.4 mW/m^2), this substrate had a COD content and a conductivity 10 times more important than primary clarifier effluent making it a good substrate for MFCs.

A better comprehension of the origins of voltage reversal in MFCs stack was elaborated. Several methods were implemented to solve voltage reversal in MFCs stack. A 12 units MFC stack was successfully integrated into with an electricity storage system. Three lithium polymer batteries were simultaneously charged by the set-up.

In addition, a wide range of substrates were tested, it was determined that the type of substrate that could be treated is strongly dependent on the type of bacteria within the anodic biofilm.

6. Outlook

The work showed that a microbial fuel cell to generate and store electricity is generally feasible. Still, there are some technical hurdles to overcome. Especially, to turn microbial systems financially applicable, it has to reach a power density of 70 W/m^2 based on the business plan described in section 4, this value was determined as a boundary condition. Therefore, the surface efficiency of Anode and Cathode have to rise (and such by reducing the surface needed, dramatically reducing the cost for the complete system). This includes questions on the waste to electricity conversion process but also electrical restrictions (e.g. improving electron assembly). These questions are not yet developed in an advanced manner.

Larger reactors would be needed to propel at least the electronic power charging and stack management for best power generation. With such an entity, one could examine if waste water purification can be realized at low energy cost. This means that no external electricity is needed, what reduces electricity investment in waste water treatment plants by 70%.

There are many questions on the engineering level to investigate but overtime improvements should be possible. The cost of materials is another point to resolve over time. By mass production such cost should decline and in view of the static nature of a microbial fuel cell the lifetime of large scale installations should be quite high and only the membranes are an element that needs to be exchanged from time to time. A larger treatment unit on the pilot scale combined with a life time assessment analysis could be very helpful as such work is not yet realized.

7. Planned future investigations

It was determined that the biofilms are key elements in the origin of voltage reversal (see section 3.3.2) in serially connected MFCs. A better understanding of this phenomenon could be achieved by studying the microbiota on the electrodes. Patterns leading to imbalance and voltage reversal could be identified which could be of use when it comes to solving voltage reversal. Despite the fact that weakened biofilms were identified as the origin of voltage reversal, the reason why a biofilm becomes disadvantaged over the others still needs to be investigated in larger MFC stacks. Such investigation could lead to preventive measures to avoid unbalanced stacks.

A series of measures were proposed and tested to solve voltage reversal (see section 3.3.4). At least, three of them successfully solved voltage reversal in a triple unit MFC stack. These measures need to be validated and implemented for larger scale installations.

12 MFCs connected in series were successfully monitored and managed by the electronic energy storage system designed and constructed for the project (see section 3.4.1). This was the demonstration that MFCs are compatible with such systems and that the energy recovered could easily be stored. Further development of these management systems could be oriented toward voltage reversal prevention. In fact, voltage reversals tend to appear at high current density, the management system could then keep the system above critical current density.

The 12 units MFC stack was run as 3 stacks of 4 units to simultaneously load three batteries (see section 3.4.2). The energy recovery from a MFC stack could be more or less efficient depending on the interconnections between the cells. An ideal circuit configuration would lead to high power density and could prevent voltage reversal. Further investigation on how MFCs should be interconnected could then be of use to scale-up the technology. The scale-up of the technology to the pilot plant could also require design improvements. In fact, the conversion efficiency of the substrate is in function of the amount of oxygen present in the anodic compartment. Working with a tubular system without headspace could prevent oxygen leakage within the anodic compartment. With a larger scale also comes the question of the volume of wastewater that such system could treat. In fact, the duration of the process or the hydraulic retention time should be characterized to reach the specifications in terms of COD removal that are currently applied to classic aerobic treatment.

Different substrates were tested and evaluated (see section 3.2) in the frame of this project. These experiments are of primary importance to determine where the MFC technology could be exploited in the future. It was determined that the key for an efficient treatment is a good compatibility between the bacteria present within the biofilm and the substrate of interest. When an MFC is run for a long time with a determined substrate, it is assumed that a natural screening occurs in the microbiota on the electrodes. In fact, only the bacteria able to metabolize the substrate and transfer their electrons to the

anode will survive. It could be of interest to characterize the duration of such screening by metabolomics analyses.

Deeper analysis of the microorganisms involved in MFCs should be conducted to acquire a better knowledge of the process. Such knowledge could contribute to prevent voltage reversals or treat new substrates adequately. The design of the power storage management system could be modified to comply with the MFCs specification specially to prevent and resolve voltage reversals.

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Annexe:

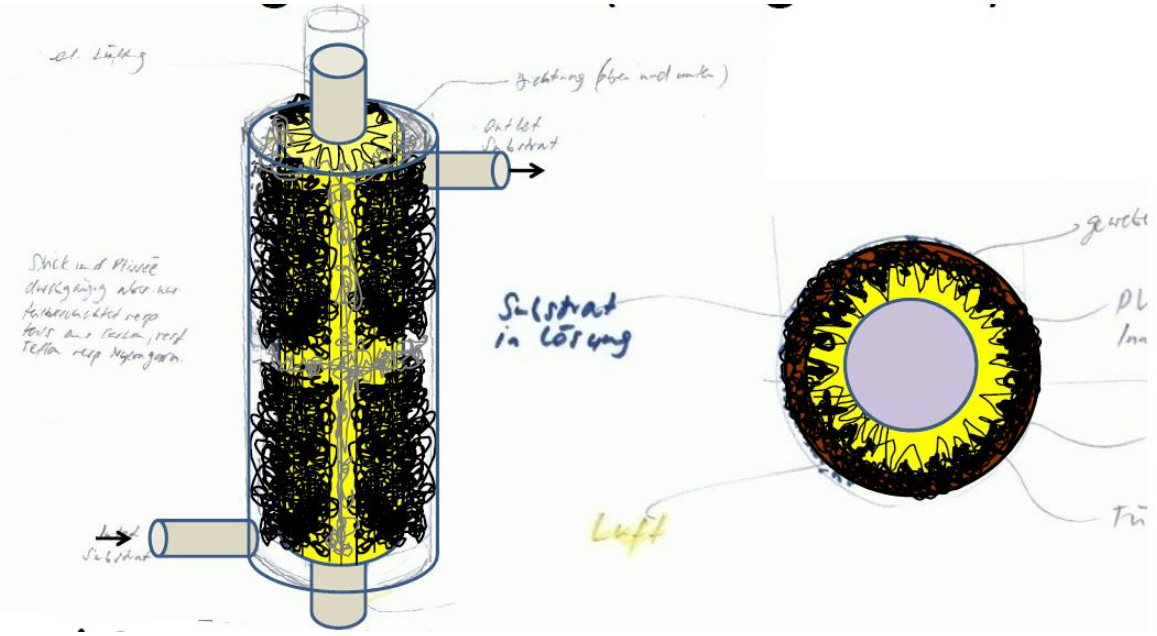

Figure A1: The design of future reactors was studied here as Tube like reactor.

Future reactor designs were studied in view of effectiveness for power generation but also ease of maintenance. The flat panel design is an option that enables well to service broken membranes and electrodes. It was also found that many material components are not available in cyclic form and the additional cost would be an additional constraint. This is particularly true for membranes and carbon anodes. However, independently of the reactors construction, the reflections showed that the MFC should be fed with effluents, such as urine, from the bottom upwards. With other effluents also sedimentation is possible and needs to be taken into account for many substrates, which contain for example sand and other none digestible components. Therefore, a MFC should be a vertical construction with relatively flat anodes and cathodes of similar sizes. For a 350W-Reactor using a high energy substrate it was found that 5 m^2 per anode, cathode and membrane is required. For a 1000W-Reactor 15 m^2.