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POWER OUTPUT OPTIMIZATION FOR MICROBIAL FUEL CELLS POWERED BY URINE

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Sommario

Nel campo della generazione di potenza attraverso fuel cell, una nuova variante è andata sempre più crescendo negli ultimi decenni: le Microbial Fuel Cells. Questa versione biologica di fuel cell si propone di produrre energia attraverso ossidazione diretta di materiale organico. Attraverso questa trattazione, gli aspetti fisici della generazione di potenza tramite MFC ad urina saranno analizzati: in particolare la condizione del catodo e la sua necessità d'idratazione, la dipendenza della potenza emessa dalla portata di alimentazione, i consumi di materia organica a diversi livelli di carico imposto, fino al problema dei sedimenti accumulatisi e allo studio di configurazioni ottimali per il funzionamento di utilizzatori low-power. Grazie ad esperimenti dedicati, è stato possibile pervenire a modificazioni efficaci del design del volume del catodo, all'individuazione della velocità ottimale di alimentazione dello stack, a incrementi di potenza, alla invalidazione di un metodo di misura (COD) per la valutazione della materia organica consumata. L'esperienza termina con l'applicazione pratica di MFC nell'alimentazione di una pompa idraulica o nell'utilizzo delle stesse come sensore di temperatura.

Parole chiave: *microbial fuel cell, urina, batteria biologica, catodo, micro porous layer, carbonio attivato, stack, potenza, sensore temperatura, struvite, COD.*

Abstract

In the field of power generation through fuel cells, a new variant has been establishing itself during the last decade: the Microbial Fuel Cells. This biological version of fuel cell offers the advantage of producing energy through direct oxidation of organic matter. Within this dissertation the physical aspects of power generation through urine will be analysed: in particular the cathode needs of hydration, the relationship between power and feeding flow rate, the relationship between organic matter consumption and loads, the filtration of struvite, and finally the individuation of stack configurations for power production. Experiments allowed defining design improvements at cathode side, to find out the optimal feeding rate and also, to validate or not the method utilized for carbon consumption measurements. The study concluded with a practical utilization of MFCs stack as a power generator for a small pump or as temperature sensors.

Key Words: *microbial fuel cell, urine, biological battery, cathode, MPL, AC, struvite, COD, stack, power, pump, temperature sensing.*

Introduction

Nowadays the development of Microbial Fuel Cells technology is proceeding in leaps and bounds if compared to its early times: electricity production is a constant, different designs and materials have been tested, feedstock of multiple nature have been processed and also various autonomous applications and robots have been successfully powered by this eco-compatible and totally renewable source of energy.

Within Bristol Robotics Laboratories, these days, researchers are following different patterns all together conveying in the single direction of making MFCs able to provide a significant and continuous power output, to be totally reliable and safe in term of avoiding failures.

It hasn't been forgotten any of the main guidelines that distinguish Microbial Fuel Cell technologies: ability to purify wastewater, capability of pathogen killing, energy production and not of less importance, water generation. All together features that made this technology a unique and promising meeting point between engineering and biology.

This thesis itself, want to bring its contribution investigating energy production levels and continuity of MFC systems processing human urine and assembled in stack configuration.

It is exactly the utilization of urine the first innovation of this study: formerly processing synthetic media TYE (Triptonyte – Yeast Extract), now one more step towards the real word utilization has been done by making human waste the source of energy of these bacterial colonies. This is also thanks to the commitment of B. & M. Gates Foundation to develop helpful technologies for third world countries ("*Urine-tricity"* project).

Innovation of MFC technology proceeded with the fist part of the dissertation: Microbial Fuel Cells weakness points were highlighted and engaged with a series of experiments. Achievements of this stage were the reduction of hydration needs, the increment of useful power output and cathode materials improvements.

After having tangibly increased the performances, the interest of this thesis convey on developing a better knowledge of these biological fuel cells. One experiment dedicated to find out the optimal feeding flow rate for MFC stack, one other to analyse urine nutrients consumption at different loads.

A research has been conducted above urine precipitations pinpointing the presence of struvite as possible valuable sub-product within MFC stack; also, devices to contrast precipitations and blockages problems have been tested widely.

Along with the second-stage experiments previously described, the extensive usage of some measuring techniques (such as COD method) allowed to evaluate reliability and effectiveness of those same methods if applied to fuel cells.

The conclusion of this project aim to finalize all the efforts made so far, improving power performance and durability, by giving a demonstration of self-sustainable system. As it should be for an autonomous system, which ideally is able to produce enough energy to satisfy its minimum vital function (e.g. by pumping itself the substrate), a small pump has been powered by capacitors charged by the power of 18 MFCs.

Despite the practical demonstration just described, powering a device may not be the only scope of application for Microbial Fuel Cells, which increased utilization, will lead to applying them to the sensing areas. One positive test has already been conducted using MFC successfully as temperature sensors.

Even if many improvements still have to be done, such as shorter time of charge, further increments of power level, this dissertation gave demonstration of the big potential the technology is capable of, especially considering the relatively short time requested by this study. It has so far to be an encouragement for future researchers and students to keep believe in the capacity and big benefits finally achievable with Microbial Fuel Cells in a much more eco-friendly power production overview.

Chapter 1 State of the art with Microbial Fuel Cell

1.1 Chemical Fuel Cells

A fuel cell is an electrical device which main aim is the production of electricity directly from sources of chemical energy, trough reactions of oxidation. Common fuel cells use different fuels such as hydrogen, natural gas or alcohols, oxidized by oxygen or other form of oxidants to generate power.

The operating principle of a chemical fuel cell is a reaction of oxidation that involves the dissociation of fuel molecules into ions and electrons, those travel from one electrode to the other, through separate patterns. Recombination of ions, electrons and other external reagents (such as O_2) into reaction products (mostly H_2O) occur at the cathode electrode.

Following, a few examples of reaction occurring within chemical fuel cell such as:

- PEMFC (Polymer Electrolyte Membrane Fuel Cell):

$$anode: H_2 \rightarrow 2\,H^+ + 2\,e^-$$

$$cathode: \frac{1}{2}O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O$$

- SOFC (Solid Oxide Fuel Cell):

$$anode: H_2 + \ O^= \rightarrow H_2O + 2\,e^- \ and\ CO + O^= \rightarrow CO_2 + 2\,e^-$$

$$cathode: \frac{1}{2}O_2 + 2\,e^- \rightarrow O^=$$

Want to show how different solution of fuel cells are available, also involving different chemical elements.

Those chemical reactions are taking place inside the fuel cell due to the presence of apposite media and catalysing agents that make the fuel reacting and give electrons out. The transfer of ions goes through a semi-permeable proton exchange membrane (PEM), that divide in two chamber the internal volume of a fuel cell (cathode chamber and anode chamber); electrons instead, travel along an external connection (conductive material) from one electrode to the other, in order to complete the reaction that was remaining unbalanced, giving this way an electrical current as a result.

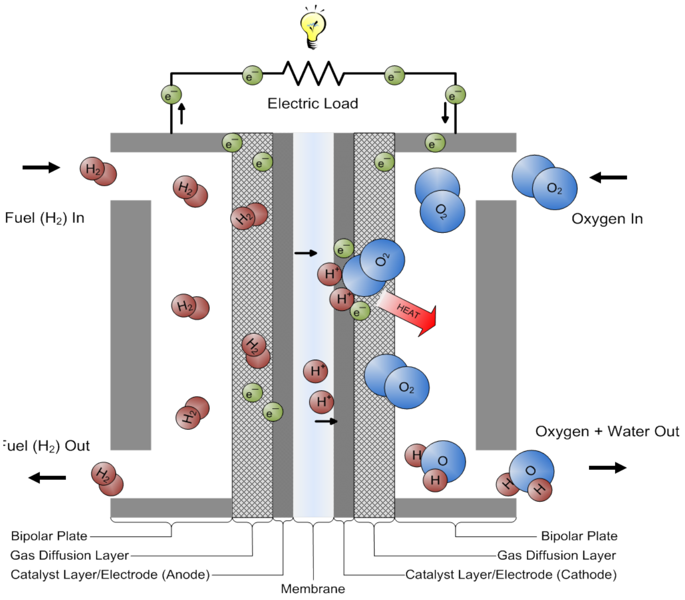

Figure 1.1 - General reactions occurring with a classic-chemical fuel cell

Fuel cells can be of various dimensions: small as batteries, or big, as internal combustion power unit. In fact, in a sort of way, they can be considered a kind of “batteries” or “power production unit”, even if their features are different.

Batteries (if rechargeable) are closed to external environment and they contain all their reactants: they could be recharged a considerable amount of times, but they need a period of inactivity (the charge) between one run and the other. Generally, instead, fuel cell needs its fuel to be introduced continuously, but they can produce energy without interruptions and their life-time it is on average longer.

Comparing a fuel cell to one power unit evidence the differences within the energy transformation process that occur into these two.

One of the main features of fuel cells is in fact, a more efficient power production process, or similarly, a smaller amount of transformation losses between the fuel and the final product, the electricity.

In a power unit powered by an internal combustion engine, the combustion process transform chemical energy of fuel into thermal energy, here it is the first loss, combustion loss. Than the expansion of heated gas is utilized to generate pressure into the cylinder chamber, a big amount of heat is absorbed by the walls, transferred by conduction at other engine parts, and thus wasted, constituting the thermal loss. Mechanical losses occur within the movement of drive shaft and the movement of electrical generator; finally one last loss to convert mechanical movement into electricity. All these losses are intrinsic the internal combustion engine, and they decrease the power generation efficiency. A fuel cell instead, doesn't have all these losses, because it has a more direct inner working: a chemical reaction of oxidation occurs without combustion (the bigger loss); through this chemical reaction it occurs the generation of electricity thanks to the electron migration from one chamber to the other one, as previously described. Even if the process inside a fuel cell involves different types of losses (activation, ohmic, mass transfer).

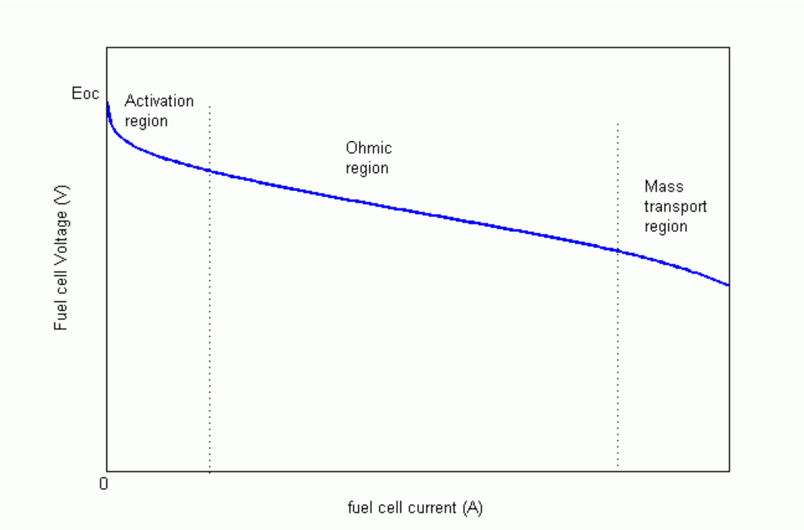

Figure 1.2 - Fuel cell occurring theoretical losses, at different currents.

The overall efficiency of a fuel cell energy production process is therefore, much higher than previous methods involving combustion.

1.2 Microbial Fuel Cells

1.2.1 Main principles

The production of energy from renewable substrates, such as biomass, is nowadays very important to create sustainable energy production and reduce global emissions of CO_2. Hydrogen and chemical fuel cells can be an important component of an energy infrastructure that reduces CO_2 emissions, if hydrogen is produced from non-fossil fuel sources. Hydrogen gas, in fact, can be biologically produced at high concentration (60%) from the fermentation of high sugar substrates such as glucose and sucrose. However, known fermentation routes can produce only 33% of the maximum potential energy from a sugar such as glucose (data from 2005). More commonly, yields of only half this amount are achieved resulting in the remainder of the energy (typically 85%) being tied up in non-fermentable or poorly fermented organic acids and solvents.

Instead of producing electricity indirectly from organic materials with biologically generated hydrogen, it is now known that electricity can be produced directly from the degradation of organic matter in a “MFC” [3].

MFC is the acronym for Microbial Fuel Cell, this is a biological version of fuel cell that contain a biofilm of few different bacteria able to oxidize directly organic matter and release electrons, transferred to the anode either by an exogenous electron carrier or a mediator (see Par. 1.2.2), and migrating trough an external connection to the final electron sink, constituted by the cathode.

These MFC are a real innovation within fuel cells, not only for their ability to process organic matter directly with higher percentage of yields, but also because of the kind of organic matter that they could process: at the actual state of the art, Microbial Fuel Cells have been tested with digested rotten vegetables, digested flies, wastewater an also human waste as urine.

Microbial Fuel Cells could than become much more promising than other traditional fuel cells (whose aim is mainly a more efficient production of energy) because of their biological side: after being power generators, they are also water generators, waste purifiers, and possibly also pathogen-killing units.

1.2.2 Microbial metabolism and electricity generation

When living creatures metabolize food to provide them with energy, they are tapping the energy of oxidation of energy-rich (means electron-rich) substances liberated, from carbohydrates for example, in reactions such as:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad (1)$$

In living cells (or in single-celled micro organism, on which we focus our attention) this process is of course a complex one involving many reactions catalysed by enzymes. It progresses through a series of intermediates involving successive oxidation-reduction changes, and in this respect resembles an electrochemical process.

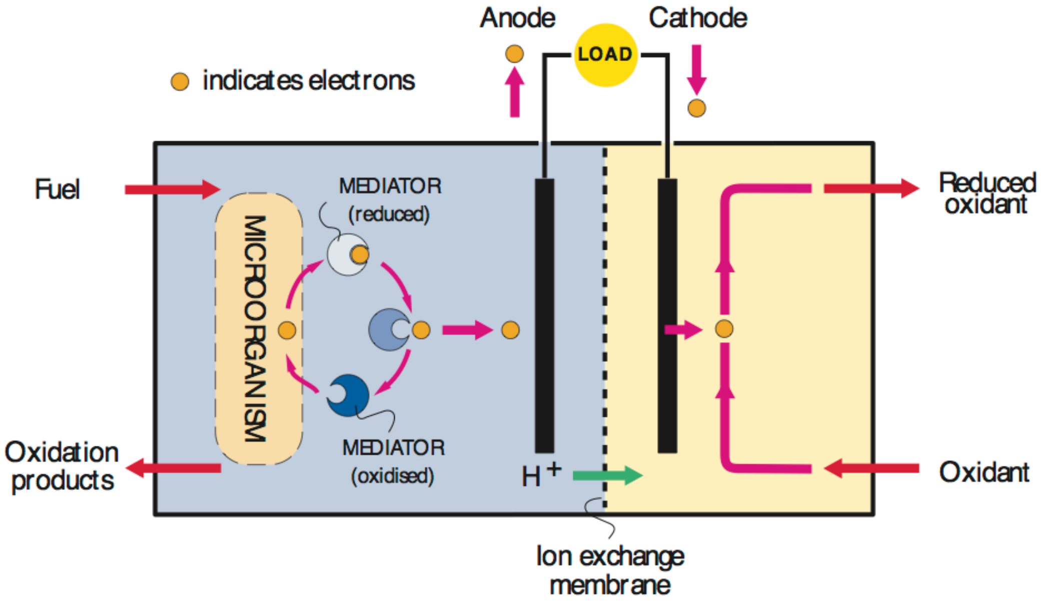

Figure 1.3 - General functioning principles of Microbial Fuel Cells

In “normal” microbial catabolism, a substrate such as a carbohydrate is oxidized initially without participation of oxygen when its electrons are released by enzymatic reactions. The electrons are stored as intermediates, which become reduced, and in this state they are used to fuel the reactions, which provide the living cell with energy for maintenance and growth via biosynthetic reactions.

The ultimate “electron sink” is molecular oxygen (dioxygen). To an electrochemist, a simplified representation of the charge separation involved in the oxidation of glucose by a whole bacterial cell would be as follows:

$$C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^- \qquad (2)$$

The large harvest of electrons is stored as reduced intermediates, but the eventual terminus in the respiratory chain is oxygen:

$$6\ O_2 + 24\ H^+ + 24\ e^- \rightarrow 12\ H_2O \qquad (3)$$

Inspection shows that equations (2) and (3) combine to give the same result as equation (1) [4].

Mediators to electron transfer

Connecting with the end of the previous page, in the absence of oxygen electrons may be diverted from the respiratory chain to other mechanisms of electrons transfer, basically aiming at conveying those from the biofilm to an electrode (which becomes the electro-generic, negatively charged, electrode) and they appear as an electric current driven around an external circuit.

Main distinction is between synthetic mediators, those where the organism is unable to exchange by itself electrons with the conductive surface, and natural mediators, those instead able to produce contacts to the electrodes.

More precisely electron transfer could be distinguished as:

- Mediated e.t.: occurring through synthetic mediators. This kind suite all those microorganisms that are incapable of electrons transfer. Synthetic mediators (dye sensitizers) have been used to facilitate the redox reactions (methylene blue, neutral red, HRQ).
- Indirect e.t.: occurring through natural mediators. For those organisms that are able to reduces oxidised molecules, as part of their metabolisms, and produce electro-active metabolites (e.g. by waste products) that facilitate the electron transfer redox reaction (sulphate/sulphide, nitrate/nitrite, ferrous/ferric).
- Direct e.t.: occurring through conductive proteins and pili (nanowires). Those organisms that are able to colonize the electrode surface and respire through this material compose this class. Those are known as anodophiles and these are two mechanisms for this.

Mediators has the peculiarity of being able to function cyclically, being reduced and than oxidized continuously (e.g. ferricyanide isn’t a redox mediator as its reduction occur as single-way, not retrieving at the initial stage).

The continuous flow

In the absence of any regeneration of substrate, the system will eventually run out of oxidizable fuel, and the electrical current flow will cease. In this mode the system is operating as a simple galvanic cell or battery, which, like a torch battery after prolonged use, will run out of " juice".

MFC, as chemical fuel cells, are also demonstrably rechargeable, because, as for the *batch mode*, the current can be restored by further and cyclical additions of substrate. After this, a continuous supply of organic fuel could also be maintained: this defines the *continuous flow* feeding, which can support the generation of electricity for indefinitely longer periods [4].

When a Microbial Fuel Cell is feed batch mode, the biofilm adapt to a lower power production, as the quality of the available substrate could rapidly decrease and biofilm will do as much as possible to maintain its minimum vital function. Studies and experiments within this dissertation (see Par. 4.3) shows batch mode MFC as a decaying system, whom is justified by a death-decline phase of biofilm population.

When instead the MFC is provided with a continuous stream of fresh substrate, the activity of the biofilm is enhanced and extremely favoured by the presence of constant fresh nutrients. This condition allows a stationary state of the biofilm, reflected with a constant and high-level power output from MFC.

It is exactly this condition of better health of the MFCs, even when connected in stack, that will be investigate in one of the next chapters (*flow rate experiment*), with the aim of even further enhance this positive effect, over power production, deriving by knowing the optimal flow rate for continuous feeding.

1.2.3 Design of MFCs

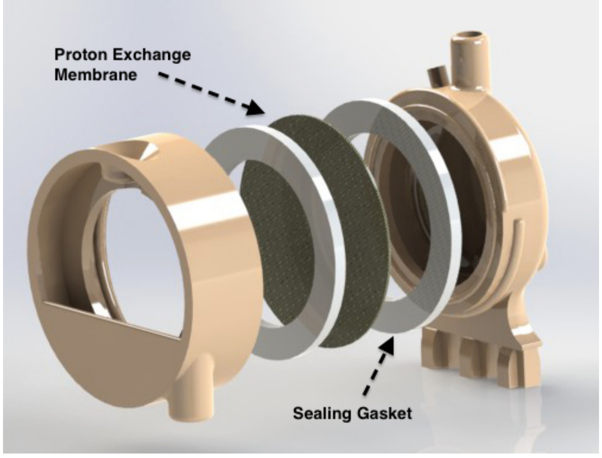

Figure 1.4 - Explosion of MFC design.

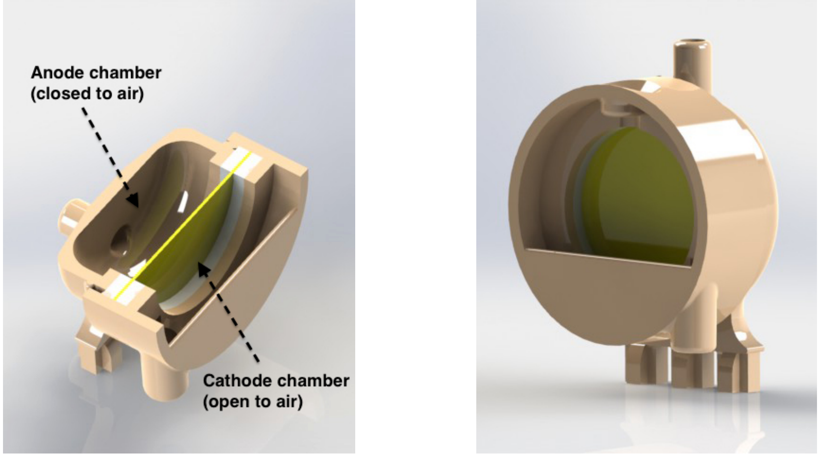

Figure 1.5 - MFC design section and perspective views

The design of this Microbial Fuel Cell was formed on the *EnvisionTec* 3D printer using *RCP30*: nano cured ceramic photo polymer material (anode and cathode chambers).

The cathode electrode was initially constructed from carbon fibre veil without any added precious metals or catalysts (PRF Composites, Dorset, UK), with a total surface area of 157.25 cm2 = 18.5 cm by 8.5 cm). Later, the adoption of Micro

Porous Layer and Activated Carbon cathodes, formed by a single layer of 25 mm diameter, reduced the cathode surface to 4.91 cm^2.

The anode was also constructed from plain carbon fibre veil, with a total surface area of 68 cm^2 = 8.5 cm by 8 cm. This is calculated from the volume within the anode chamber, which is 6.25 ml by ratio factor of 10.8 cm^2/ml = 67.5 cm^2. This ratio 'figure' comes from an established fuel cell configuration where the area was 270 cm^2, divided by the volume of 25 ml = 10.8 cm^2/ ml. Anode chamber volume is 6.25 ml. Carbon veil was 30 g/m^2 which is considered as thick, so more fibres means greater surface area means more biofilm. The carbon veil is then folded 5 times to fit into the MFC chamber. Folding is preferable to cutting the veil to fit - cutting the veil affects the pressure, conductance and resistance. The maximum number of times you can physically fold is five or you won't have enough space to fit it.

The anode and cathode are then held in place with titanium wire electrode and fitted one each into the cathode and anode chambers. To fit this size of fuel cell the wire had a diameter of 0.559 mm. A length of wire was cut, threaded through and then back out and then twisted to hold together.

For the cathode the positioning of this wire was also useful for holding the veil in the open chamber. The gaskets to seal the anode chamber and the membrane were cut from silicon sheets (~1 mm thick). The proton exchange membrane was then cut into a circular piece of ~ 35mm. The Anion Exchange membrane (*AMI-7001, Membranes International Inc.*) was preconditioned for 24 hours in a 5 % NaCl solution at 40 °C for membrane hydration and expansion. Once preconditioned can be stored 'wet' at room temperature – membranes must remain hydrated. In the anode section where the electron pokes through this is sealed with wet black silicon to prevent leakages from this port. Port for anode electrode wire is not shown in the figures above, because locate on back top, it is a tiny opening to allow wire to come out.

The second folded carbon veil (plus titanium wire) is then placed within the open cathode chamber. In the anode the carbon veil (plus titanium wire), gasket and then membrane are place within and screw together with the cathode chamber. Once assembled the fuels cells was checked for leaks by pumping water through.

Chart 1.1 - MFC main dimensions chart

Anode Carbon veil	68 cm^2 = 8.5 cm x 8 cm.
Anode chamber volume	6.25 ml
Cathode Carbon veil or Cathode AC & MPL	157.25 cm^2 = 18.5 cm x 8.5 cm 4.91 $cm^2 = \pi\ (2.5\ cm)^2/4$
Anion Exch. Membrane	9.62 $cm^2 = \pi\ (3.5\ cm)^2/4$
Titan wire diameter	0.599 mm

1.3 Urine as a valuable fuel

Urine: why exactly urine? Simple: it is the most common biological waste that a person can generate, without any cost, virtually in any situation on earth.

Thanks to the ability of the liver to enhance the water solubility of organic substances absorbed, all the compounds in excess are removed from human body by urine.

In the last decade, urine has been considerate [7] a valid source of nutrients, recyclable as a valuable product, if properly treated. Studies from NASA Bioastronautic Data Book [14] shown the composition of urine made by 158 different components, broadly categorized as electrolytes, nitrogenous compounds, vitamins, hormones, organic acids and miscellaneous organic compounds. Among those, only 68 constituents have individual maximum concentration exceeding 10 mg/l, adding up to 36800 mg/l in average urine. The other 90 compounds total approximately only 250 mg/l.

For engineering purposes, and therefore this study, a simplified list of components would give a sufficient accurate analysis, taking into accounts 42 of the 158 compounds composing 98% of the total solute concentration in urine.

Organics compounds in urine could be approximated by the equation $C_2 H_6 N_2 O_2$, and Chart 1.2 shows the bigger categories of those elements, allowing the partition in inorganic salts, urea, organic compounds and organic ammonium salts broken down into content of carbon, nitrogen, oxygen, hydrogen and organic sulphur.

Chart 1.2 - Summary of C, N, O, H and organic S in typical urine.

	Amount [mg/l]	C (12.0) [mg/l]	N (14.0) [mg/l]	O (16.0) [mg/l]	H (1.0) [mg/l]	S (32.1) [mg/l]
Inorganic Salts	14157	100	0	1877	7	0
Urea	13400	2680	6253	3573	893	0
Organic Compounds	5369	2466	1211	1231	347	134
Organic Ammonium Salts	4131	1630	659	1576	266	0
TOTAL	37057	6876	8123	8257	1513	134

Though not the only important reason, it is the big availability of urine to make it such a good candidate for powering MFCs. We have to consider in fact how such a device could become useful in any emergency situation: in the middle of a desert or during an excursion on the mountains, or simply in the everyday life when outdoor and without any "plug" to recharge our favourite mp3 player or mobile phone.

But also, lets consider any isolated villages from not developed countries such as African nations or India: imagine, how helpful and life-changing could be one day this technology for all the 1.200.000.000 (billions!) of people in the world that are still without electricity, 20% of the world populations. We have to consider the benefits of using a device that would produce electricity, while purifying a waste and possibly killing diseases transmitted by urine.

Thanks to their small dimensions (such as the design utilized within this thesis, or even smaller ones), a stack of them could easily be embedded in public toilets and contribute at the energy efficiency of new buildings, with the most simple and available waste: urine.

Principles and methods utilized to measure organic compounds in urine (COD) are explained within the appendix (*A.2COD measurement*)

1.4 MFCs stack

The experiments of this study were conducted over a group of 25 MFCs, with same design as descripted above.

Connecting two or more MFC as a serie of volumes, in which the outlet of the first anodic chamber become the inlet of the second and following, is resulting in a fluidic connection between the two (or more) and is called *stacking* of MFCs.

A main stack was built for the purpose of this study, connecting fluidically 18 MFCs.

The 18 MFCs were located one over the other, held by clamps in a vertical column and connected to each other fluidically by short plastic tubing. Feedstock was provided from the bottom of the stack to the top, by connecting the inline to the first anode chamber inlet.

A secondary stack was built with the remaining 6 fuel cells, with the same structure, in order to conduct minor experiments and verifications.

In the following script it will be so distinguished between the *main* and *secondary* stack.

Connecting as stack a group of fuel cells means that they will “work” as team consuming the feedstock provided: this will flow from the first on the line, through all of them, till reaching the outlet of the last MFC in the stack. This architecture will have advantages, as the reaching of higher waste treatment (COD reduction), but also disadvantages, as decreased power due to lack of total insulation between units [12], or higher probability of clogging.

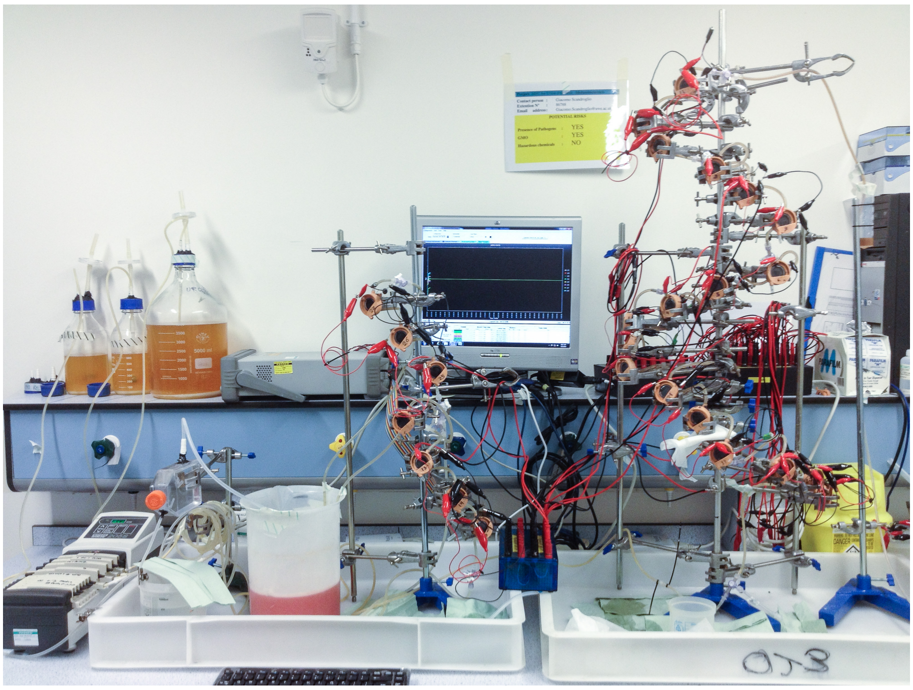

Figure 1.6 - Final configuration of Main Stack and Second Stack within laboratory view

From the electrical point of view, MFC were connected in parallel by group of three, constituting 6 units called *triplets*. This choice allowed having more powerful units, by raising the current according to the first law of *Kirchhoff,* for the purpose of powering applications.

Still concerning the powering side, stacking is also the winning choice to reach higher levels of power, if units are connected as series. Indeed, precedent studies have been proofing the better effectiveness of stacking of units, instead of the increment in anode volume, if increasing the power is desired [12].

Sampling ports constituted by plastic *T-connectors* were placed at the beginning of the stack, and after every three MFCs (triplet), allowing to take samples of the substrate going in and out each triplet.

A peristaltic pump (*Mod. Watson & Marlow mod.205 S*) was used to push the feedstock through the stack, allowing a precise control of the flow rate.

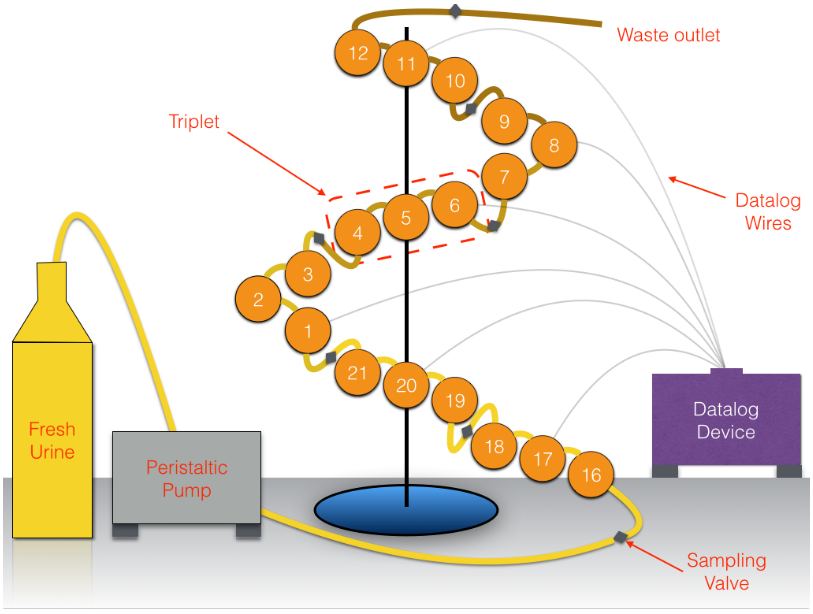

Figure 1.7 - Model of the main stack, highlighting units and main components.

From hydraulic retention time measurement (HRT, explained within Procedures chapter), it has been possible to measure the empty volumes of each MFC utilized within this study. These volumes were intended as operative volumes, in order to quantify the amount of substrate allowed in each one; they were ideally the resulting from subtracting the anode electrode volume from the empty-chamber anode volume.

Chart 1.3 - Volumes of all Triplets and Main Stack parts

Average Volumes Measured	
	[cm^3]
All Stack of 18 MFC:	64,68
	62,64
AVG Triplet:	9,14
Triplet 1 (16-17-18)	9,99
Triplet 2 (19-20-21)	10,89
Triplet 3 (1-2-3)	8,35
Triplet 4 (4-5-6)	8,49
Triplet 5 (7-8-9)	8,48
Triplet 6 (10-11-12)	8,63
AVG MFC:	2,381

AVG short pipe:	0,922
AVG long pipe:	1,826

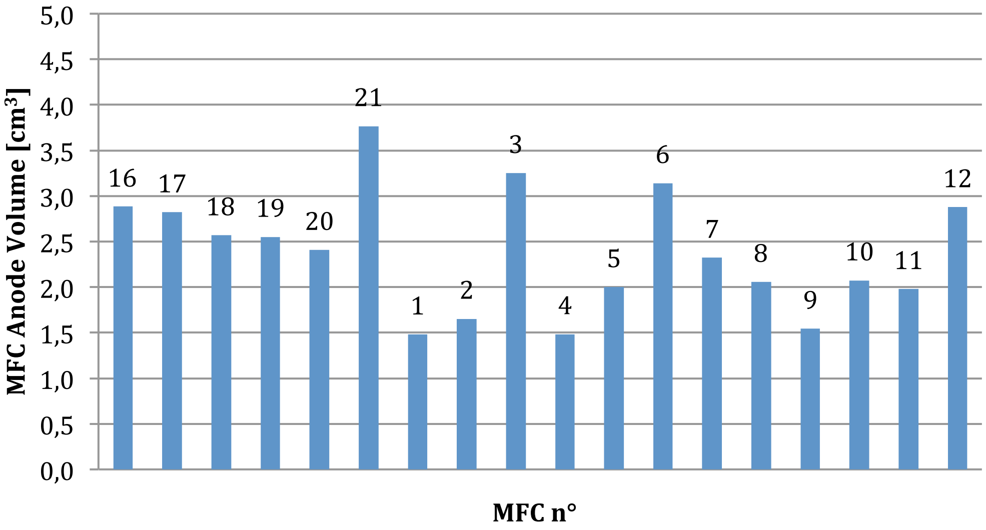

Figure 1.8 - Differences between empty volumes inside each anode chamber (label shows number of each MFC.

It has to be considered that few of the MFC showed a very different internal operative volume (see Figure 1.8). Starting from the assumption that initial dry volumes of each MFC should be identical, this is already an alert about how effective could be the accumulation of debris within anode chamber. Consequently it has to be taken into account that the condition of initial equal internal volumes for the anode, cannot be stated.

Chapter 2 Power level enhancements

2.1 Power output improvements by using different cathodes

2.1.1 Introduction

The research of increasing power output, with MFC, goes also through the innovation process of using new and better materials. As cathode has been many times proofed to be a limiting factor of MFC, any modification over it can largely affect performances: the completion of oxidation-reduction reaction goes through the cathode and its property of conductivity and ability to repel water.

With the following chapter it will be analysed the influence over power output of an increased quality of materials for the cathode. First carbon veil (CV), than micro-porous layer (MPL), till activated carbon cathode (AC) will be replaced over eighteen MFC and tested through polarizations and observing eventual water formation, expected effect of a complete reaction.

Initially, MFC cathodes were built with carbon veil sheet, folded many times till reaching a compact multi-layer rectangular cuboid shape.

Carbon veil, so folded, was occupying the whole cathode chamber (open to air chamber) and was connected with the activated membrane just by the first layer of folded veil.

A first evolution of cathode design was done utilizing a new micro porous layer cathode, made by a single layer carbon tissue, with a completely new micro-structure.

It was cut in a circular shape, in order to match the size of exposed-to-air membrane surface. The whole cathode surface was now on contact with the membrane surface.

Despite the big improvements obtained with the power output, MPL cathode was kept for a short period of time (one week), due to the availability of an even newer material.

The second step of evolution has been done with activated carbon cathode. Also made by a unique layer cathode, its contact with the membrane was enhanced by plastic ring, ideally increasing contact pressure between cathode and membrane, aiming to a better function of the cathode and avoid flooding under it.

2.1.2 Materials and Methods

Before describing the procedure of the experiment, here is a short summary of elements utilized to produce each material and respective methods used for production. Even if costs are not included in the description, it has to be taken into account also the cost factor of the material, in order to aim at mass or large-scale production of MFC, for big stacking and power production. Still, all those solutions of cathode materials are to be considered as low cost if compared with Pt, even with high surface area and high catalytic activity, which makes AC a good candidate to its replacement.

Carbon Veil cathode

Carbon Veil cathode is based on a catalyst free carbon fibre veil with a carbon loading of 20 g/m^2 (PRF Composites, Dorset, U.K). This was used as initial condition for the cathode (and anode) electrodes.

Analysing the material with SEM images, a filamentous and not organized texture is highlighted, structure that scarcely enhance the surface area due to its lacks of cavities.

Micro Porous Layer cathode

The MPL based cathode material consisted of nano-sized carbon black powder and carbon cloth as a supporting matrix.

Electrodes (two layer carbon cloth/carbon black electrode) were made from a gas diffusion layer (GDL) and a micro porous layer (MPL), forming a two-layer structure. The GDL was made from carbon cloth treated with 30% by weight polytetrafluoroethylene (PTFE) (Fuel Cell Earth) to ensure wet proofing. PTFE treatments are also required to give the material some strength and make its less porous. The MPL comprised a mixture of above mentioned nano-sized carbon black particles (Vulcan XC-72R, Cabot Corporation, Stanlow, UK) with a non-ionic surfactant (Triton X100, Sigma Aldrich), deionised water and PTFE (60% emulsion, Sigma Aldrich). The MPL formed a thick paste that was applied to the GDL surface and then heated to 343°C to allow liquefaction of the PTFE and penetration of the carbon nano-particles in the porous structure of the GDL. Due to the carbon black nano-particles, the MPL consists of a high surface area, which is optimal for increased oxygen reduction [13].

Activated Carbon cathode

AC is nonetheless an improvement of MPL cathode too, basing its structure on same components, but more precisely engineered.

From the literature [6] here is the procedure of fabrication of AC-based cathodes prepared via pressing: the pressure was ≈ 2200 psi and ≈ 3300 psi applied for 20 minutes and the material was dried at 70-90°C over night. The best hydrophobic treatment (Polytetrafluoroethylene (PTFE) content, 10-40%wt), AC loading (7-171 mgAC cm^{-2}) on the cathode performances was achieved at PTFE content of 10% and AC loading higher then 43 mg AC/cm^2. In that case, the pressure applied on the material was roughly 3300 psi.

Methods

A group of 18 microbial fuel cells was initially operating with carbon veil of 20 g/m^2 weight and size 18.5 cm by 8.5 cm, resulting in a total surface area of 157.25 cm^2. This was folded five times, forming a three-dimensional cuboid shape that fit into the cathode chamber. In order to keep cathode veil on site, folded and to have a vector for electrons, titanium wire of 0.559 mm was used, threaded through and then back out and then twisted to hold together. This design was tested and running for approximately one year.

The first material substitution was between CV and MPL cathode.

From MPL sheet, circles of 25 mm diameter were cut, resulting in a surface area of 4.90 cm^2. MPL cathodes were than threaded with titanium wire (same as above), in a point close to the edge, and than twisted to hold together.

The diameter of this single-layer cathode was chosen in order to cover completely the exposed surface of the membrane. Because of its positioning at close contact with the membrane, it was demanded to use small plastic bars, positioned inside the cathode chamber, to apply pressure to the MPL sheet and maximize the contact area.

After having replaced all the cathodes for the main and secondary stack, feed flow was restored. Hydration at this stage was still on continuous flow: cascade disposition of the MFCs was allowing drops to fall cathode by cathode and, by regulating water flow rate, all cathodes were kept hydrated (since power output without external hydration was still too weak). Main stack was left on normal operative conditions for few days, so MPL cathode could activate and be fully operational participating in the water mass transfer reaction.

Unfortunately, power output increments due to MPL cathode could not be quantified within this study, as at the same time, main stack was subjected also to substrate changes: artificial urine was substituted by real human urine, which greatly contributed to power increment, making impossible to quantify merits of MPL of urine. This was justified by the impellent need of power increment within the context of a different experiment.

After few days of operations under real urine the system adjusted to its new steady-state, so the carbon cathodes could be considered fully activated. A polarization was performed on all the 18 fuel cells of the main stack, in order to quantify the initial power output condition of MFC with urine and MPL cathodes.

After having installed MPL cathodes for just a relatively short time, and because the main aim of other experiments running at the time was to have the highest power output as possible, a new cathode material, Activated Carbon cathode was installed on MFCs.

The AC cathode was cut in circles, this time slightly larger than 25 mm diameter to allow it to fit and being hold in place by plastic composing the cathode side of a MFC, right over the membrane and cathode side gasket. Better surface contact between cathode and membrane was so provided and even improved by installing over the cathode a plastic ring that fit within the diameter of 25 mm and push on the outer case of the fuel cell, providing enough resistance to hold the cathode on site.

After having substituted all 18 MPL cathodes with new AC cathodes, feed was restored.

Following the instructions provided by the AC producer, hydration was now reduced from continuous dripping to manual. The premise of utilization of this AC cathode, as for MPL, was of self-hydration due to its ability to promote water mass transfer and of hydrophobicity on the inner surface, incentivising hydration of membrane.

Despite knowing this, it was observed that voltage was still fluctuating: increasing every time manual hydration was applied, and than decreasing by the time. Manual hydration was than maintained twice a day.

A few days were given to the new material to fully activate and than polarization was performed.

2.1.3 Results

Carbon Veil and Micro Porous Layer comparison

The substitution of cathodes was inserted within a more articulate experiment concerning other aspects of benefits from MFC stacking (pathogen kill rates).

A big boost in power production was required at the time, and therefore materials and substrate followed a fast escalation not always well documented with data and analysis.

Within this first step of MFC technological evolution, both a change with substrate (TYE to Urine) and cathode material (CV to MPL) was applied and not enough data were collected to differentiate the two contribution. It has to be clearly stated that therefore it isn't possible to quantify performance benefits brought by adoption of MPL cathodes.

Despite that, since the technological improvement took place, it is considered important to describe the two materials features and their differences, highlighting researcher's understanding of a justified attribution of power increment.

To understand how cathode could contribute improving the power output, it is important to understand its role in water transport and management within MFC architecture. The cathode should work as catalyser, and also keep the membrane completely hydrated in order to reduce ohmic resistance.

Water at the cathode needs to be optimized aiming to create a clear 3-phase interphase and enhance the cathode reactions. Although it wasn't ever the situation of this work, it has to be considered also opposite situation: an excess of water could create flooding phenomena and penalize the cathode overall performance.

The water fluxes taking place at the cathode are mainly due to electro-osmotic drag, diffusion (evaporation), and back-pressure. Moreover, the water production due to the oxygen reduction reaction (ORR) is an important contribute that cannot be neglected.

The electro-osmotic drag is a phenomenon of water transport due to the drag of water molecules exercised by the protons during their migration through the membrane. The electro-osmotic drag generates a water flow from the anode to the cathode [5].

Another water flow is due to diffusion (evaporation) and mainly driven by a different water concentration gradient between two sides (internal and external) of a permeable porous material (membrane, MPL, carbon cloth and carbon veil). Water diffusion flow can be easily described using a Fick's diffusion law applied to a one-dimensional homogeneous domain as the membrane. The variation of diffusion coefficient varies significantly between the different CV and MPL, due to different materials porosity, hydrophobicity and nano and micro scale properties.

More than others, the back-pressure (convection) flow produces a water flow from the cathode to the anode that depends from the local different pressures close to the membrane and from the wettability/hydrophobic characteristics of the cathode materials. [5].

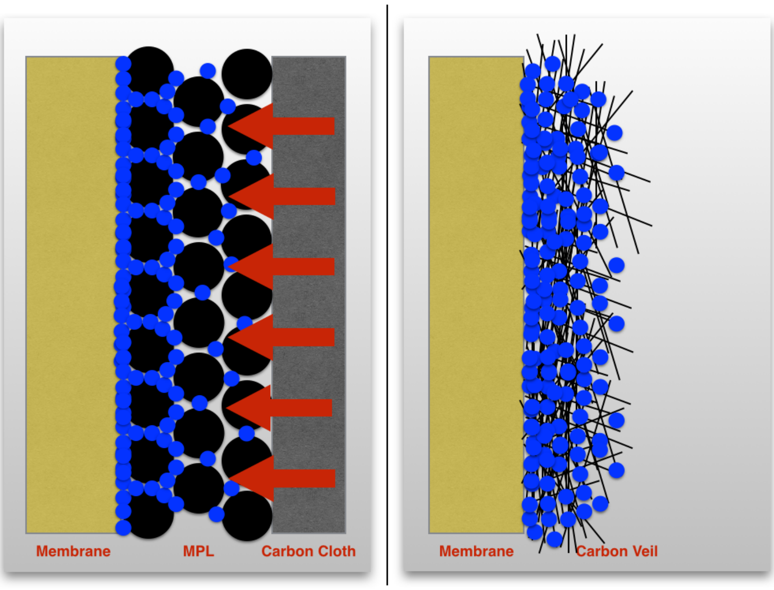

Figure 2.1 - Microscopic view of CV and MPL cathode and scheme of their interaction with water molecules.

Analysing more in details the very different properties and microscopic structure of these two materials:

1. Possibly because of its far bigger (considering all the cavities) surface area and its higher power output, MPL cathode showed within other studies a higher power density [µW/m^2] than CV cathode.
2. Analysis conducted with SEM microscope (as scheme shows in Figure 2.1) showed a very different microscopic structure between the two: the MPL, thanks to its micro-porosity, has a bigger active surface area, which will result in higher surface to catalyse cathode reactions and therefore increasing MPT.
3. From recent studies [5] it has been proved that there is no real need of a multi layer cathode as the most active part of it is the first layer in direct contact with the membrane and also that multi-layer packed together could disadvantage the reactions.
4. Finally but not less important, Also the different water management due to different material characteristics could have an important effect on the

> performances: the MPL had very hydrophobic surface characteristics (θc ≈ 140-150°) and very low pore size (lower than 1 micron) and the carbon veil having completely opposite characteristics (hydrophilic and pore sizes in the order of mm). The contact angle on the carbon veil is practically impossible to measure, in fact when a water droplet is gently deposed on the surface, the water is completely adsorbed by the carbon surface. Those different characteristics led to a strong back-pressure flow in the case of MPL utilization that keeps the membrane completely hydrated, lowering the ohmic losses, and clear and defined 3 phase interfaces zone where the ORR is enhanced (Figure 2.1 also shows water interaction). At the contrary, the water formed or transported for electro-osmotic drag and diffusion get adsorbed by the thick carbon veil who does not create any back pressure flow. The carbon veil acts as a "sponge" adsorbing all the water that remain soaked on the surface. The water remains trapped on the carbon veil creating not defined 3 phase interfaces zone and favourable conditions for flooding phenomena lowering the power output. It is possible also that the membrane on the cathode side could be partially dehydrated leading to increase in ohmic losses [5].

For all these reason it is clear that, even if data were not clearly recorded[1], big improvement could be attributed by usage of MPL cathode within Microbial Fuel Cells technology.

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[1] *Even if the contribution of urine and MPL over power output was not differentiated, polarizations were recorded, making possible to quantify an overall power increment of*

Micro Porous Layer and Activated Carbon comparison

MPL Microbial fuel cells, running with urine were subject to polarization test.

Chart 2.1 - Results from MPL polarization

MFC n°	**16**	**17**	**18**	**19**	**20**	**21**
MPT [µW]	31.25	44.89	31.34	30.37	43.20	28.64
P.d. S [µW/m^2]	4596.2	6601.5	4609.4	4466.4	6352.7	4211.3
P.d. V [µW/m^3]	5.0e+6	7.18e+6	5.02e+6	4.86e+6	6.91e+6	4.58e+6
R [Ω]	2k	1k	2k	2k	1k	2k
MFC n°	**1**	**2**	**3**	**4**	**5**	**6**
MPT [µW]	63,41	93,26	27,73	44,48	37,35	107,99
P.d. S [µW/m^2]	9324.5	13714.6	4077.6	6541.4	5492.1	15881.3
P.d. V [µW/m^3]	1.01e+7	1.49e+7	4.44e+6	7.12e+6	5.98e+6	1.73e+
R [Ω]	2k	1k	4k	2k	2k	1k
MFC n°	**7**	**8**	**9**	**10**	**11**	**12**
MPT [µW]	61,61	39,64	80,11	23,90	44,36	25,80
P.d. S [µW/m^2]	9060.8	5828.7	11780.4	3515.1	6523.7	3794.3
P.d. V [µW/m^3]	9.86e+6	6.34e+6	1.28e+7	3.82e+6	7.10e+6	4.13e+6
R [Ω]	1k	1k	1k	1k	1k	1k

Second kinds of single-layer cathodes, with Activated Carbon were tested on the whole main stack (18 MFC).

Chart 2.2 - Results from AC polarization

MFC n°	**16**	**17**	**18**	**19**	**20**	**21**
MPT [µW]	55,23	81,61	52,89	65,11	44,6	117,65
P.d. S [µW/m^2]	8122.1	12001.5	7777.9	9575.0	6558.8	17301.5
P.d. V [µW/m^3]	8.84e+6	1.31e+7	8.46e+6	1.04e+7	7.14e+6	1.88e+7
R [Ω]	1k	1k	3k	1k	2k	1k
MFC n°	**1**	**2**	**3**	**4**	**5**	**6**
MPT [µW]	91,02	198,32	123,79	96,61	126,26	86,82
P.d. S [µW/m^2]	13385.3	29164.7	18204.4	14207.4	18567.6	12767.6
P.d. V [µW/m^3]	1.46e+7	3.17e+7	1.98e+7	1.55e+7	2.02e+7	1.39e+7

R [Ω]	2k	1k	1k	1k	1k	1k
MFC n°	**7**	**8**	**9**	**10**	**11**	**12**
MPT [μW]	115,37	68,121	81,61	30,57	77,66	83,24
P.d. S [μW/m^2]	16966.2	10017.8	12001.5	4495.6	11420.6	12241.2
P.d. V [μW/m^3]	1.85e+7	1.09e+7	1.31e+7	4.89e+6	1.24e+7	1.33e+7
R [Ω]	1k	1k	1k	3k	1k	1k

Power density was also calculated, referring to both to the anode total surface area:

$$P.d._S = \frac{MPT}{S_{Anode}} \left[\frac{\mu W}{m^2}\right]$$

And the anode “empty” volume:

$$P.d._V = \frac{MPT}{V_{Anode}} \left[\frac{\mu W}{m^3}\right]$$

A further improvement within cathode material brought under comparison MPL and AC cathode (AC/PTFE cathode), this time fully supported by polarization data.

Following, two of the best performing MFC were taken into account and their complete polarization output is represented within Figure 2.2 and Figure 2.3 below.

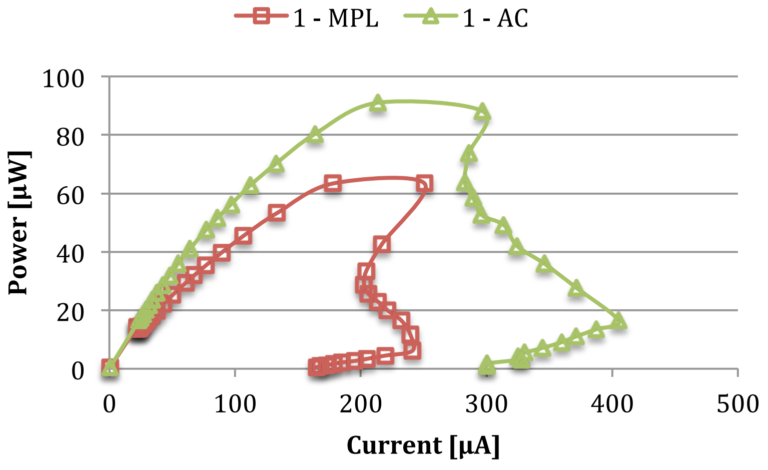

Figure 2.2 - Comparison between MPL and AC cathode polarizations over MFC 1

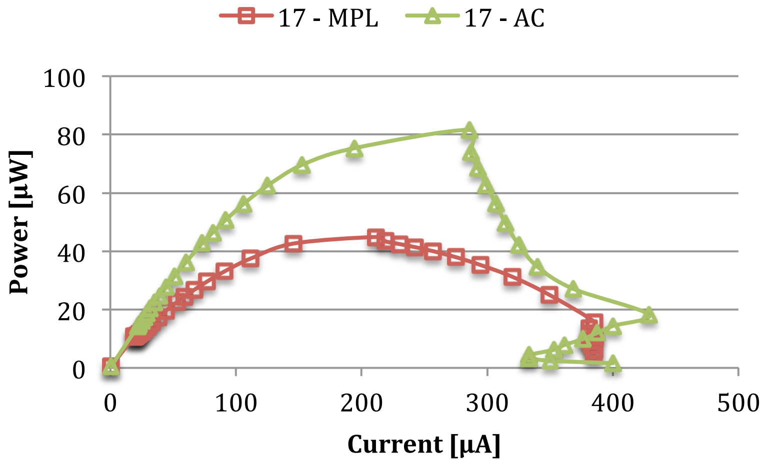

Figure 2.3 - Comparison between MPL and AC cathode polarizations over MFC 17

With these two similar materials, it is not anymore a matter of completely innovate the structure of the layer (see disordered fibres structure of CV compared to pores of MPL, Figure 2.1), but instead of improved knowledge of the material itself, that allow transferring these technological progresses into increased power output.

There are two main parameters affecting the performance of cathode catalytic layer towards oxygen reduction: conductivity and porosity. It is well known fact that *oxygen reduction reaction* (ORR) takes place at three-phase interface solid-gas-liquid or otherwise catalyst-air-electrolyte. The solid phase provides electron transport and catalyses the ORR. Gas phase ensures oxygen diffusion and the liquid phase is responsible for proton supply.

In AC/PTFE catalytic layer the PTFE drops are covered with AC particles and due to the PTFE fibres forms a compact body. PTFE provides also hydrophobicity and forms gas channels. The hydrophillicity of the AC particles helps the proton diffusion. AC conductivity is responsible for the electron transport, therefore changes in carbon layer conductivity and porosity will influence the cathode current output [6].

In Figure 2.4 below, it is possible to see MPT values over 18 MFCs installing first MPL and than AC cathodes: it has shown clearly a new big improvement in term of power output, thanks to cathode improvements (nonetheless again indicating for these MFCs the cathode as a limiting agent).

Increase in power attested at average of 36% increment, with few peaks also reaching over 60% (excluding MFCs bad performing for general weakness of the whole MFC).

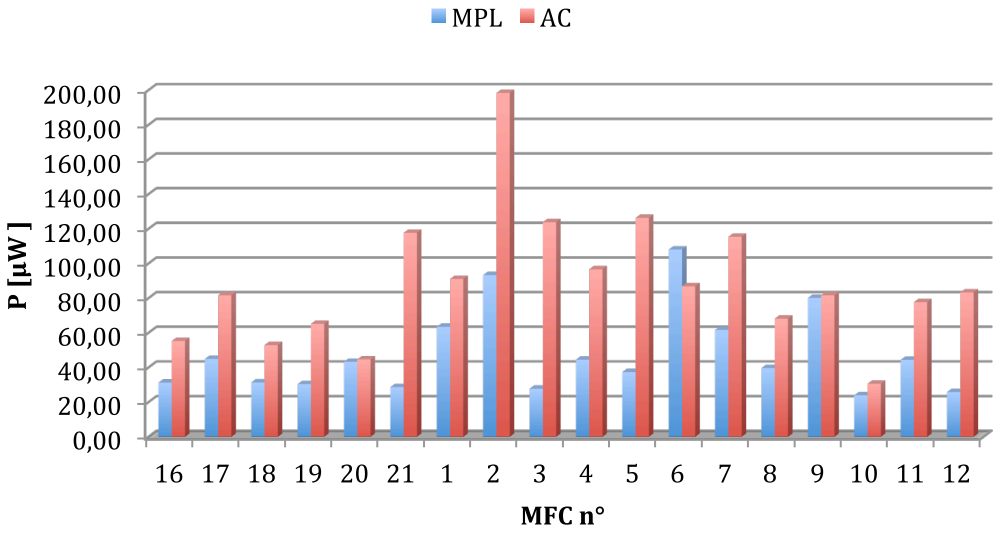

Figure 2.4 - Comparison of MPT with MPL and AC on 18 MFCs

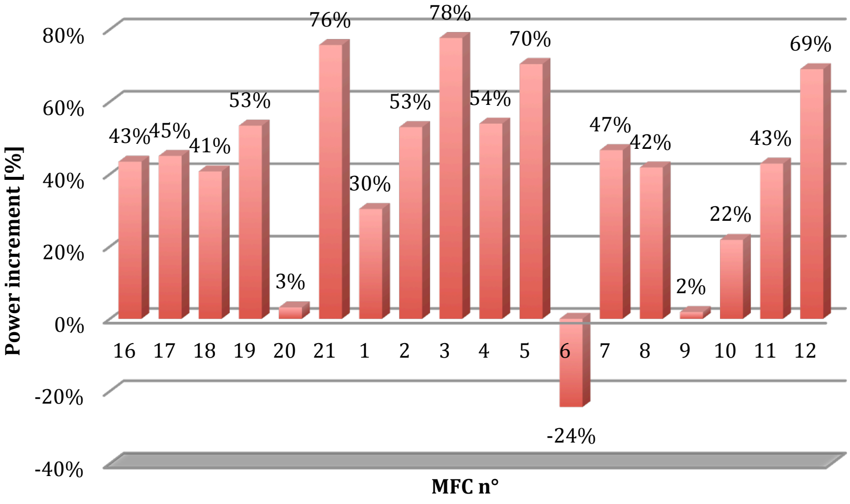

Figure 2.5 - Increment of maximum theoretical power from MPL to AC cathode, expressed as percentages

Reasons of this power increment could be searched in the improvement of fabrication methods for this material. Recent studies explained better the properties of this activated carbon based cathodes, and indeed successfully highlighted as important constructive variables the pressure applied to AC particles and the treatment temperature [6].

In general, in fact, AC is not a good conductive carbonaceous material due to the high surface area that certainly enhances the ORR, but due to the particle-to-particle distance, reduces the capability of electron transfer through the materials and increases the material ohmic resistance. Therefore, the rise in cathode performance shown in Figure 2.4 and Figure 2.5 could be explained by a better increase in contact among the AC particles, if compared with the one on MPL, and consequently a reduction of materials ohmic resistance.

Also a better management of treatment temperature, in order to optimize hydrophobic properties of the material, would bring its contribution to power output. The PTFE is used within this cathode as a hydrophobic and binding agent. A clear link between performances and PTFE transition behaviour was underlined [6], as different production temperature will influence the surface and the way the particles attached to each other. At different temperatures, PTFE experience a change in phase, in fact after roughly 150°C, the PTFE tends to expand and to vitrify making the surface smoother. At the contrary, additional increase in T, led to PTFE melting and consequently producing a much higher roughness. Mesoscale range skewness at both low and high filter showed [6] highest number of small pores with temperature treatment at 200°C (10 nm-1μm) and generally tends to increase with temperature (2-8μm). At the contrary, for the macro scale range, the large pores decreased with temperature rising.

Hydrophobic properties, as explained for MPL within the previous chapter, are fundamental to the MFC functioning, as a correct hydrophobicity will enhance membrane hydration, other fundamental parameter for increasing the power produced.

It has to be noticed that the constant increment of power verified on most of the MFC tested after the introduction of AC, is one more proof of consistency in the improvements.

Therefore with this section, it has been possible to understand the reasons that brought increment between AC and MPL carbon cathodes. Proofing that a better understanding of the phenomena undergoing, will lead to a smarter development of materials and design.

2.1.4 Conclusions

This fundamental experiment allowed to underline once more a well-known as key and limiting factor of MFCs: the cathode, and how it is possible to obtain big improvements with it.

Along this chapter an evolution of cathodes within MFC has been shown, together with an increase in power output. Starting from a more rough material which was allowing electron transport and a certain level of low water mass transport; than a totally different geometry with microscopic pores and a single layer for the MPL, allowing a much higher conductivity, water repulsion and improving drastically the ORR and the hydration of the membrane, previously occurring very scarcely and often leaving the membrane dehydrated.

Finally adopting AC cathode, with its steady and repeated improvements in power, allowed to stop the attention on parameters not very well considered before such as designing pressure and temperature, demonstrating that attention on constructive parameters within material is the funding and starting point for build a consistent improvement in the technology.

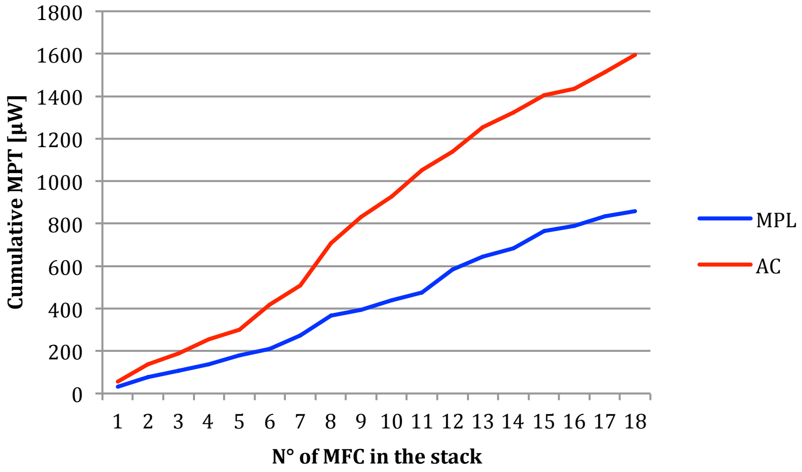

Figure 2.6 - Comparison between cumulative MPT with different cathodes, on 18 MFC.

Within this section of the study, the involved MFCs recorded both a final increment in power of more than 85% total (see above), and also a drastic reduction of external hydration, decreased from continuous dripping flow to very infrequently hydration (>48-72h), possibly due only to opposing agents such as room temperature and ventilation systems, contributing in cathode dehydration.

Chapter 3 Power stability enhancements

3.1 Reduction of hydration rate needed at cathode side

3.1.1 Introduction

Power output stability it's a very important requirement for any device demanded to power real life applications: if the fuel is constantly supplied, than the power produced should keep the same constant level.

In the case of MFCs, power is desired to be constant while fresh urine is fed. With MFCs, power output stability means also a quick feedback on health conditions of the bacterial biofilm at the anode, or oppositely any malfunctioning feedback with design components (wires, membranes and cathodes).

It is the very starting point of any experiment: in order to conduct any analysis over MFC, and highlight for example the influence of a varying parameter, it is necessary to being able to assume normal stable operative conditions, confirmed through power output stability.

Considering this all, hydration experiment has been designed to fulfil the requirement, and assure constant conditions over the main stack, for further experiments.

Also, hydration experiment, showed with great clarity the dependence of power output with the hydration conditions of cathode-membrane; this time the analysis was conducted observing the operative output (real output), confirming once more the results and consideration done within the cathode substitution chapter and its power values from polarizations.

The voltage records shown below in Figure 3.1 represent an average condition of the six triplets before the hydration experiment.

The voltage of each triplet reached its peak every time the cathode was hydrated, following fast decreasing to its minimum within a relatively short period of time. On average, less than 4 hours were required to the voltage to go halved, and reach its minimum within 12 hours.

All the triplets were following the same pattern (Figure 3.1), just differentiating by the maximum levels reached, due to their position along the feeding line.

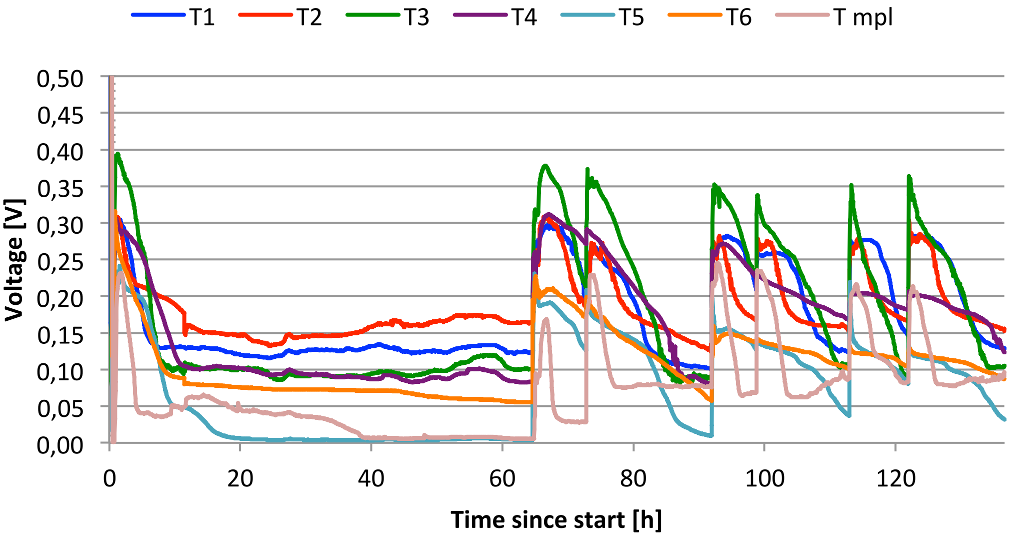

Figure 3.1 - Voltage output over 18 MFC before Hydration experiment (period from 26/4 to 02/5)

Also the big low plateau, displayed at the beginning of Figure 3.1, represent the behaviour of the main stack with absence of manual hydration (during weekends for example). All the minimum voltage levels were reached shortly and than held with no variation or increase, clearly displaying a consistent dehydration of cathode and membrane and its strong dependence with low power output.

This poor performance has to raise the problem of MFCs self-sustainability and ability to produce water to complete the reaction at the cathode side. In order to mitigate this, manual hydration was regularly applied to MFCs, by spraying or squirting tap water over the cathode tissue; this operation produces only short lasting effects, due to exposition of cathode to room temperature and ventilation system, which were causing fast evaporation of water from cathode and subsequent dryness.

In the attempt of preventing this, cathode volumes have been sealed with two different methods, Parafilm plastic film and later, some appositely designed acrylic lids. These two solutions were initially tested on two different well performing triplets; other triplets were left uncovered.

Great results were immediately obtained, maintaining a high power output for a longer time, and slowing the process of evaporation from cathode; this led to the choice of trying plastic lids on all 18 MFCs composing the main stack. Power output and time required from power to decrease after manual hydration were monitored for a week, in order to ensure the consistency of the improvements achieved.

After having also repeated the experiment from the very initial point, it was possible to come to conclusion that the simple sealing of cathode chamber from air, was an effective design evolution that allowed power output to stay stable for much longer intervals of time, contrasting with success external agents concurring to evaporate the eventual water production.

Also, a proof of better hydration condition of the membrane was the increased minimum power produced by each triplet, globally giving an overall higher minimum power.

3.1.2 Materials and Methods

The main stack was initially hydrated manually with a frequency of 2 times per day; hydration was consisting in squirting 1-2 ml of cold tap water on each cathode. No water was left stagnating on the cathode surface, due to itself vertical orientation. Activated Carbon cathodes were already installed.

Flow rate was kept constant at 1 RPM (11.04 ml/h), and loads were calculated based on the most recent polarization (Polarization 24/04):

Chart 3.1 - Loads applied to each triplet during Hydration exp. and most recent MPT values.

N° TRIPLET	1(bot)	2	3	4	5	6(top)
Max Power Point [µW]	308.3	295	473	445	233	172
Resistance [Ω]	305	407	407	205	1000	1000

Due to their very good power output, triplet 3 (MFCs 1-2-3) and triplet 4 (MFCs 4-5-6) were chosen for a preliminary test.

“Parafilm” plastic film, a deformable, thin plastic foil, utilized to seal and insulate, was applied over cathode chambers of MFC 1, 2 and 3, paying attention of not leave any major leakage.

The preliminary test got positive results voltage output over the following 48 hours decreased much slower than other triplets, causing the positive effect of a big increment with power (compare the Area Under Curve), solely noticeable on the covered cathodes MFCs.

Preliminary test passed, the following step involved the design of a hardcover lid for cathode chambers. An acrylic thin plate, with half-circular geometry, was appositely designed to fit the internal boundaries of the cathode chamber, and seal it from external atmosphere. Just a small central hole of 1 mm diameter was left, to allow external manual hydration.

Oxygen could anyway fill the cathode chamber, passing through the upper hole existent within the cathode-side walls (which was initially meant as inlet for continuous flow hydration).

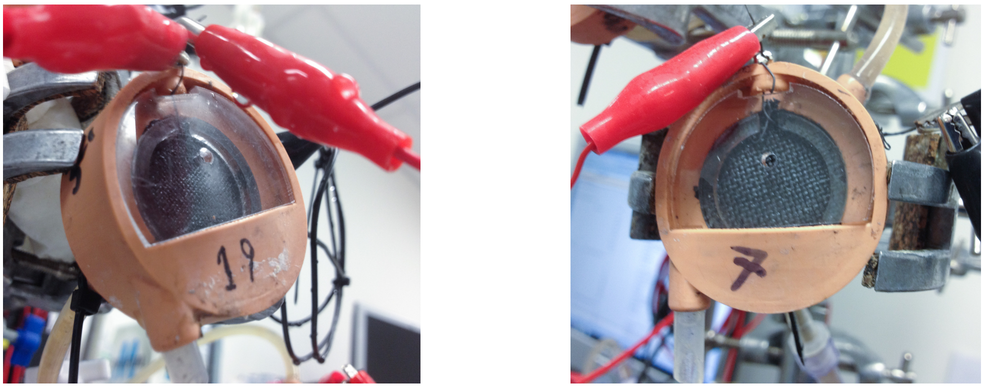

Figure 3.2 - Acrylic lids closing cathode chambers before and after being sealed.

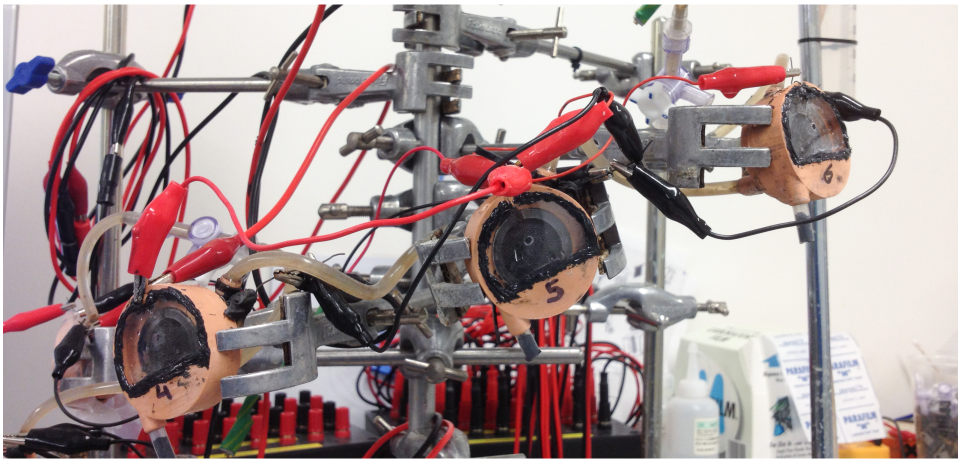

Figure 3.3 - Acrylic lids over all cathode chambers, finally sealed with black silicon.

Acrylic lids were applied to triplet 4, Parafilm was maintained on triplet 1. Power output was monitored for two days, improvements with duration of high power output were maintained, lids were than installed on all main stack MFCs (Figure 3.3). Sterile black silicon was used in small amounts to glue the acrylic lids to the inner walls and to better seal the cathode chambers.

After having all lids installed, feedstock was provided fresh with a daily base. Manual hydration was applied every 12 hours and, its effect on power output monitored through PicoLog logging system, measuring the voltage of each triplet.

A higher hydration rate was also tested (every 2 hours): it was desired to see if possible to reach a saturation level, and if so, at which value.

Monitoring of power output was continued for more than one week at the hydration rate of one every 12 hours.

After having the results confirmed for multiple days, experiment had been repeated from the beginning. The aim was to show the usage of closed chamber cathode as the direct responsible for increased power stability and also, to test power output levels with less frequent hydration rates (once every day). Lids were than unglued from the MFC shell and cathodes again exposed to air.

Interesting details about water production at the cathode side were noticed at this stage of the experiment.

Main stack was left without lids for approximately a week, on normal operative conditions, in order to see if the initial condition of fast decreasing power was again occurring.

After that, all 18 MFCs were again sealed with black silicon and also modelling rubber was used to close front and upper holes of cathode chamber, with the attempt to preserve even longer the effect of manual hydration. The front hole was opened and closed every time was manually hydrated.

Hydration rate was reduced from every 12 hours to every 24 hours or also every 48 hours and stability monitored over a week.

3.1.3 Results

Power output stability enhanced

Every power output of each triplets has been analysed on a daily base, measuring how long the triplet with covered cathode was able to hold a high level power output (between 75%-100%). An example of this could be found in Figure 3.4.

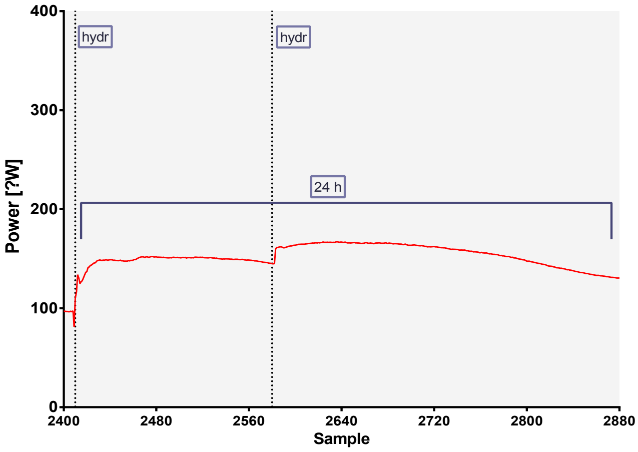

Figure 3.4 - Example of triplet power output and stability after having lids installed. Vertical bars show the exact time of manual hydration.

Comparison has to be done with Figure 3.1 that represent the initial average voltage for each triplet.

First of all it is very clear, especially for the healthier MFCs, the big importance of hydrated cathodes in order to allow optimal power production.

Assuming that the "quality" of urine was not among the variables of this experiment, because it was kept always as fresh as possible (and also because as real, an exact constancy was not possible), it has been observed many times that a MFC with decreasing voltage was experiencing a bad working cathode causing dehydration of the membrane.

The dehydration of the membrane is something very negative when aiming at maximizing power production. The three-phase interface that need to occur between the membrane and the cathode, in order to allow the ORR to be completed, when water mass transport is missing, will miss the liquid phase responsible for the proton supply, nullifying the power production.

It is considered at the end of this experiment, that a small water transport was occurring at the cathode side, as proofed by Figure 3.9 later, but this was too small amount if compared to the counter effect of evaporation imposed by the ambience high temperature during summer season (measured temperatures around 27-29°C constantly) and by diffusion mass transfer imposed by different ambience humidity.

Acrylic lids were so meant as a durable design modification to protect cathode layer foil from ambience temperature and external agents, slowing down any possible evaporation process and also keeping a higher humidity in the cathode volume.

The main effect of adopting these lids was the increased power stability. A high level of voltage is sustained by MFCs for a longer time, and this is directly causing a positive increment of power produced (see how area under curve, which represent the power delivered, has greatly increased in Figure 3.5-Graph 3 and Graph 4).

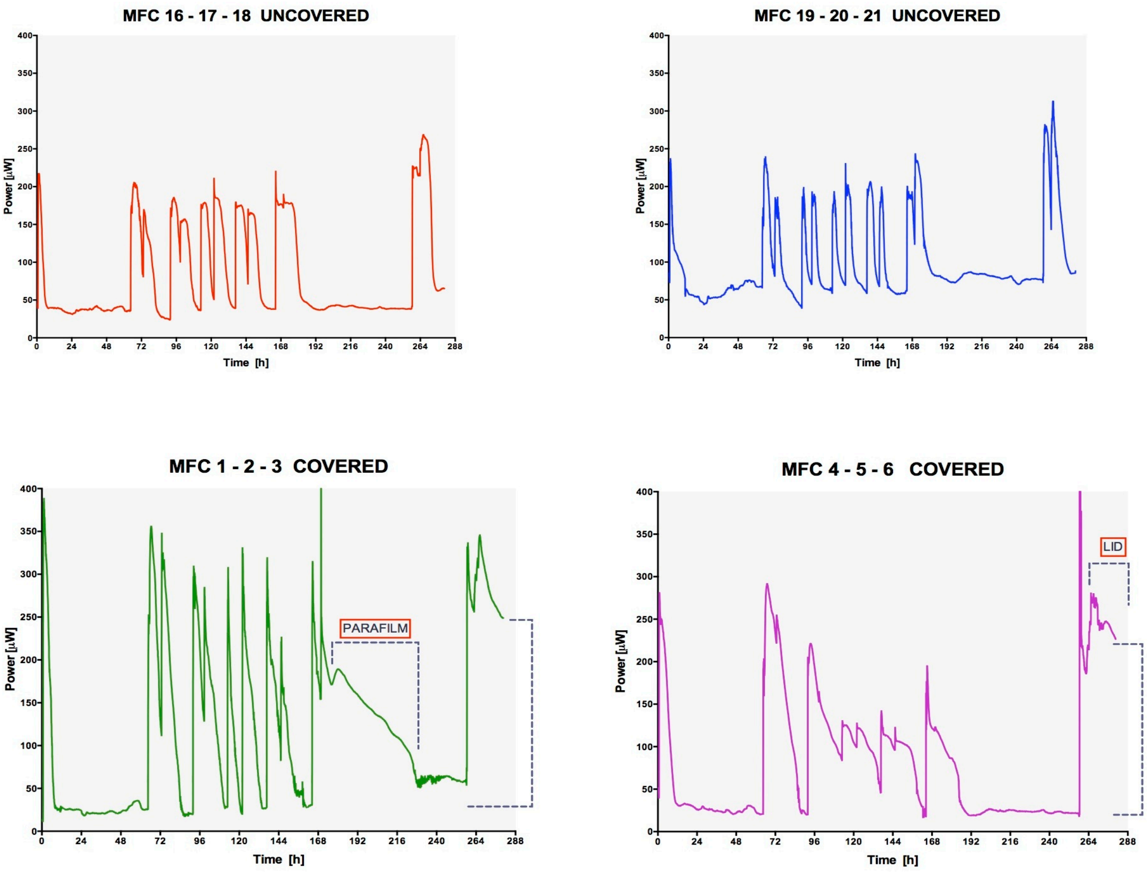

Figure 3.5 - Voltage output before Hydration experiment for each single triplet

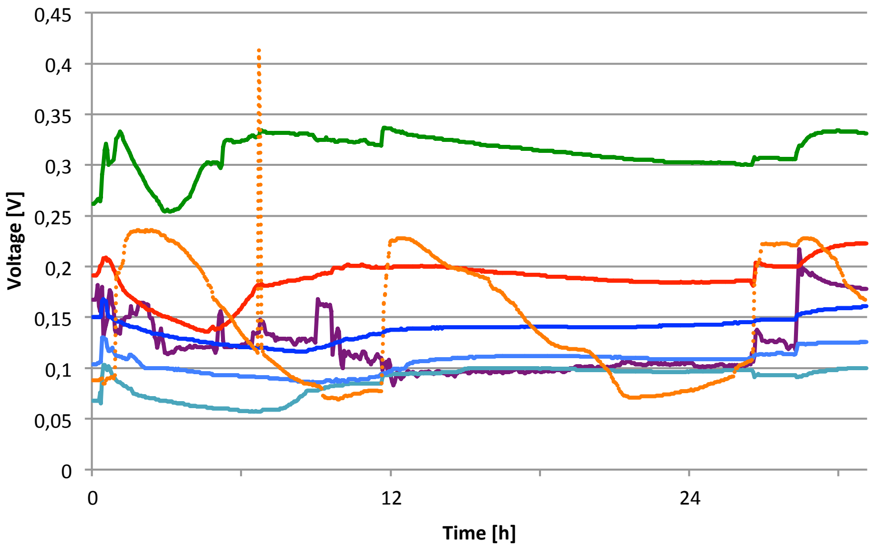

Figure 3.6 - Stable voltage output from main stack, over interval of >24 hours, after having lids installed. Reference period: 10/06 to 12/06.

Figure 3.6, is a clear example of the results achieved: MPL triplet (orange line within the graph), left without lids as control, was following the hydration pattern proper of the initial condition of the triplets. Hydration effects were fast fading within less than 12 hours. Covered MFCs oppositely, were keeping the same level of voltage for at least 48 hours, well over four times longer the uncovered MFCs.

Moreover, even when hydrated, the small increase in voltage was the demonstration that optimal level of hydration was very close.

Finally, even if power was decreasing after many hours, it was still possible to notice that the minimum power delivered was also increased (See Figure 3.5-III e IV). This is due to the ability of a close volume to keep cathode fibres wet and to keep any possible water evaporating, inside the cathode volume itself, working as a reservoir of humidity for the cathode and membrane wetness.

Further confirmation of this improved stability comes from the reduction in frequency of manual hydrations: from every 3-4 hours to every 12 hours or also more. No higher hydration rate was required.

Previous statement was demonstrated with a small test, run within the experiment: very frequent hydrations (every two hours) were applied to a well performing triplet. Those are reported within Figure 3.7.

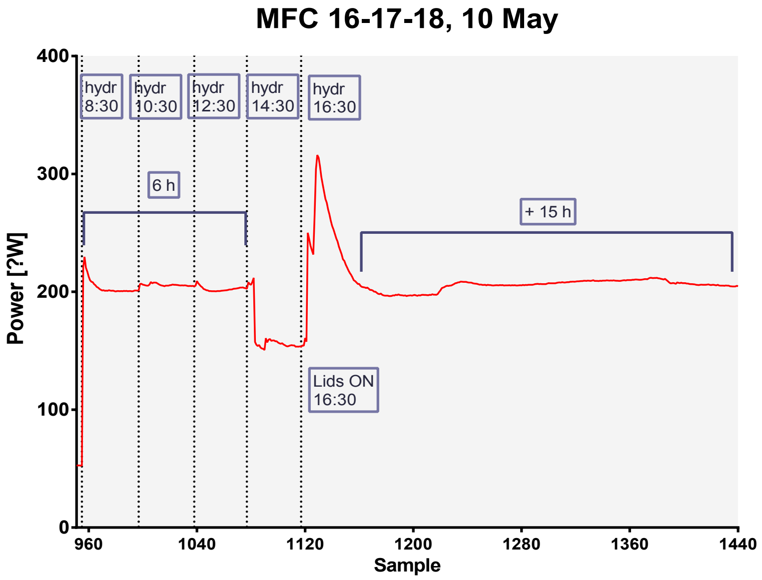

Figure 3.7 - Effects of multiple very frequent hydrations on power output and stability.

Here it is possible to notice that the new configuration of sealed cathode volume was able to keep the optimal hydration condition: even adding frequently fresh water the power output was not varying from its operative maximum.

This situation was then conserved after the last manual hydration, for much more than 15 hours and remarkable is the level of power around 200 µW constantly. Same results were reported for the other triplets, with decreasing average power for the last triplets on the stack.

Considering all this positive result listed thus far, first phase of hydration experiment was considered positively ended. A second run of the experiment in any case planned, in order to obtain verification of lids installation benefits.

Cathode finally self-sustainable and needless of hydration

Very positive effects of lids installation/sealing of cathode chambers, encouraged the researcher to test the system further, pushing this to the limit of lack of hydration.

The intention so far was to verify the maximum time past without a sensible decrease in power output attributable to dehydration of cathode/membrane.

Hydration experiment was completely repeated, starting from uncovered stable MFCs, with the aim of confirm the previous results, this time however, hydration rate was brought to its minimum frequency. From a rate of twice a day to one every 3-4 days.

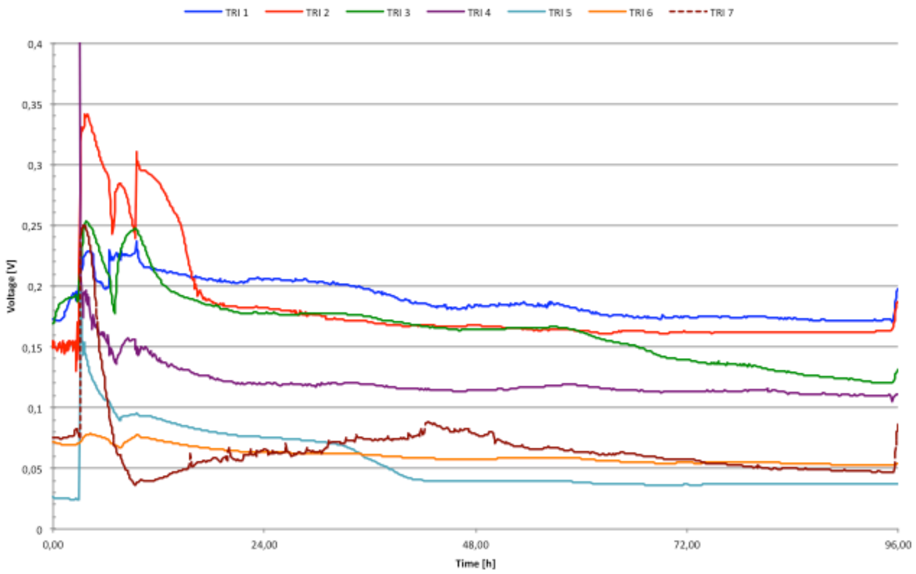

Figure 3.8 - Power output of main stack, keeping a very stable power production over almost 4 days without hydration.

Second phase of the experiment was able to fully confirm the improved hydration condition of cathode and membrane, translated with a longer-lasting power output. Closed-chamber cathode here demonstrate its essential contribution to MFC good operative condition; what could be further added is the evidence that possibly, the cathode reached a condition of self-sustainability in term of hydration.
This event of self-hydration (together with a increased internal humidity of the cathode chamber) is indeed the only possible explanation to such a prolonged stability, demonstrated within the second run of the experiment and exemplified within Figure 3.8.

It is possible to see how Triplet 7 (control without lids, represented with dotted brown line), although starting from a respectable voltage, is immediately affected by dehydration and its voltage drop within the known 12 hours. All the other triplets instead, kept an almost steady power output reaching the 96 hours without hydration (4 days!).

This result is also the proof that, preventing the atmospheric evaporation of liquids from the cathode, it is possible to see a tiny trial of self-sustainability, allowing the small amount of water produced at the cathode side, even if microscopic, to be enough to sustain ORR reaction and therefore an acceptable power output. As demonstrated when unsealed for repetition and with control triplet, all the descripted above is solely merit of the acrylic lids over cathode chambers.

Chart 3.2 - Example of measured power output from main stack when not hydrated for more than 48 hours. As shown by different measuring dates, levels are consistent. A) 01/07/13: not hydrated since 2 days. B) 28/06/13: not hydrated since 2 days.

A) 01/07/13	T1	T2	T3	T4	T5	T6	Tmpl
Load [Ω]	300	300	300	500	1000	1000	666
Voltage [mV]	144	179	85	111	121	76	102
Power [mW]	69.12	107	24	24.6	14.64	5.8	15.6

B) 28/06/13	T1	T2	T3	T4	T5	T6	Tmpl
Load [Ω]	300	300	300	500	1000	1000	666
Voltage [mV] h 8:00	139	209	108	118	125	83	178
Power [mW]	64.4	145.6	39	27.8	15.6	7	47.6
Voltage [mV] h 12:00 (fresh u.)	159	236	112	126	128	85	178
Power [mW]	84.6	185.6	41.8	31.8	16.4	7.2	47.6
Voltage [mV] h 16:00 (hydr)	194	235	132	189	142	100	242
Power [mW]	125.5	185.3	58	71	20	10	88

Water production of MFC

Speaking about microbial fuel cells, water production at the cathode side is a very important phenomenon. It is in fact, one of the main aims of this technology and also the theoretical sign that the reaction with external oxygen has been completed successfully, that the membrane is correctly hydrated and the excess of water has been expelled.

Ideally, OR reactions within fuel cells cathodes, would lead to formation of water as explained before, acting as a self-sustaining, regenerating component of the reactions. At the current stage, only few designs have been proved to be producing evident amounts of water; all the rest instead, had required the introduction of water from external source.

This is why, even though water production at the cathode wasn't a primary target of this study and therefore it hasn't been investigated deeply, the appearing of water drops on some of these MFCs, worth mentioning the topic.

Considering specifically the condition of the MFCs utilized for this study, water production at cathode side was never been experienced before applying acrylic lids.

As previously said, it is plausible to assume that a role was played by room temperature and ventilation system, highly affecting the tiny amount of water produced by this size of MFCs.

It has to be said also that an initially poor knowledge of the phenomena connected with cathode-membrane interaction, didn't allow to focus on the importance and necessity of having the cathode very tight pressed against the membrane, to promote as much contact as possible for ions flow from membrane to cathode.

Once the cathodes were substituted, they were just slightly pressed against the membrane by the contact metal wire; not all the areas were in very well contact with each other and just after the introduction of acrylic rings (see Figure 3.9), the pressure increased locally at the edges. With the adoption of acrylic lids, along with the usage of acrylic rings pressing the cathode on the membrane, it is plausible to say that condition where much more in favour of water formation. Pictures below in Figure 3.9, shows few cases, verified on different MFCs, of drops formation.

Figure 3.9 particularly shows as point of droplets nucleation the external side of the cathode surface, precisely the one where pressure has been applied by plastic ring. Therefore the contact between membrane and cathode may have been optimal in this case, and weak or tiny every time water wasn't forming properly.

This production has been found after the first stage of hydration experiment, once lids were removed and cathode chambers unsealed with the aim of repeat the experiment. Because in all the cases, manual hydration was not done since at least 48 hours and other MFCs, equally hydrated were showing all dry cathode it is possible to state that water had been generated autonomously by the MFC.

Figure 3.9 - Demonstration of random water drops formation over cathode with different MFCs.

3.1.4 Conclusions

With this experiment it has been extensively highlighted how the adoption of covering lids over cathode chamber could be an incisive design modification that allows efficient prevention from dehydration. Hydrated cathodes and therefore membranes, gives a longer-lasting and much consistent power output.

A result produced within this section is surely gaining the stability of MFC power output, now without doubt considerable steady and durable (much longer than before). This, as explained at the beginning was a fundamental requirement to proceed with the experiments of this dissertation.

Second improvement is for sure the related strong decrease, at the limit null, request of external hydration. Hydration without lids is proved to be necessary every 3-4 hours, now instead, MFC could provide flat power output for more than 24-48 hour, and easily go further.

Also this stage of the dissertation, allowed to focus on possible design improvements in order to aim at a constant water formation. In future, attention must be given to ensure a perfect contact between cathode and membrane, maybe using new designed cathode shell, or also by hot pressing together the two, in order to slightly melt them on the contact surface.

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3.2 Problem of debris accumulation (struvite) within MFC stack

3.2.1 Introduction

Struvite is a phosphate mineral contained in urine, mainly composed by magnesium ammonium phosphate; it precipitates forming sandy deposits and crystals when the urine gets alkaline. Its chemical formula is $NH_4MgPO_4 \cdot 6H_4O$, and it is the most common mineral found in urine precipitations. Struvite crystals could appear with colours from white to yellow or pink.

This section is interested to treat struvite as both a wanted and unwanted sub-product of the process: struvite accumulation within the narrow piping could cause flow blockage, consequent increase of pressure over connectors or membranes, and finally will led to leakage in the system. Struvite, on the other hand, it's a valuable product itself, utilized in agriculture as a fertilizer and therefore extracted from urine as main product.

Since the aim of this project is far different from the production of struvite, the main target of this section was to find a consistent solution to prevent system clogging, possibly separating urine and struvite before it get into the MFCs.

Furthermore, since struvite, even though known, was never investigated before, analysis of residual were carried on to verify that struvite formation was indeed occurring inside MFC stacks.

Starting from fresh urine, with dissolved struvite in solution, the precipitation was occurring within the following 24-48 hour, favoured by the increment in pH from acid (pH 5.4 – 6) to neutral or even alkaline. Precipitation was occurring where the fluid was more abundant, helped by higher probability to have nucleation points. This place was generally the storage bottle. At slow flow rate, these sediments would probably stay inside the bottle, but still constitute a problem for the inlet of the suction; at fast flow rate, they were transported through the main stack and were accumulating within the anodic chambers.

Different options were designed and tested in order to create a settling volume, before the main stack inlet, that would allow struvite crystals to be collected there instead of proceeding further.

The principle followed was to give the liquid enough room to slow down, and allow the gravity force to win over viscous forces, dragging the heavy particles on the bottom. Possibly establishing a laminar flow at the surface level and extracting separated urine from there.

A first attempt was done with a long rectangular plastic bottle: a certain amount of struvite were collected there allowing analysis, but still the rest of the flow was too much in a state of agitation, allowing some crystal to proceed within the anode chambers.

A second attempt was done with a long acrylic cylinder, with a bigger volume compared to the first, and a disposition of inlet and outlet respectively at the far bottom and at the far top, obliging any entering particles to travel all the way up, against gravity, in order to escape the settler and reach the stack. The bottom of this settler also had a sampling port, where easily extract the material precipitated. This design has been the one better working and guaranteeing an efficient extraction of struvite. Despite this, due to the high volume of the same, not always experiments requirements were allowing a high HRT for this volume.

Finally, a third solution was made by a floating thick polyester plate, kept elevated by feet, where the piping was threaded, facing the bottom. This floating sustain was intended to keep the inlet drawing close to the surface level, where was more likely to have the lower concentration of sedimentation.

Also this solution worked fine, especially with high flow rate, were it was hard to set a fix position for the inlet, because of the urine level fast changing within the tank.

Many samples were than collected, dried and, a good amount of them, selected because of different sources within the stack, to be analysed with EDX ESEM analysis, in order to characterize their average composition. A clear confirmation that struvite was being obtained, come from those analysis, opening also further roads and usages for microbial fuel cells.

After having tried to solve the problem of struvite accumulation within the fuel cells stack, this study was able to show some preliminary solutions about how to contrast the phenomenon of clogging and transport of particles.

3.2.2 Materials and Methods

Devices tested

The first device tested as a struvite settler was made with a flat rectangular bottle (see Figure 3.10), which was drilled on the bottom lateral side to allow inlet pipe to be glued with black silicon. One other hole was drilled at the top, on the opposite side, and a rubber pipe with smaller diameter was sealed into it, meant as an outlet.

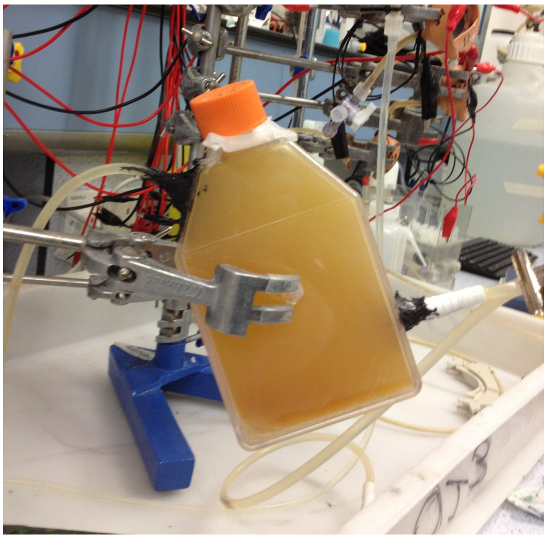


Figure 3.10 - First design of struvite settler. Wrongly engineered.

This shape of bottle was initially chosen because of the V-neck and the tap that would convey debris settling at the bottom, and also, allow easy removal. Unfortunately the design of the cap wasn't evaluated correctly and it wouldn't sustain the pressure of the liquid if positioned upside-down. For this reason a vertical positioning was required, not allowing the V-shape to be used. Anyway, device was tested, connecting it at the entry of the stack; so all the sediments coming from the source bottle were trapped there.

The second design of settler being tested was made with a long acrylic pipe, with the internal diameter of 5 cm and the overall volume of 500 ml developing in length. Two strong rubber stoppers, with conical-section profile, were used to seal both top and bottom of the cylinder.

The top one had a smaller hole, to allow inlet rubber pipe to goes through, still maintaining the internal volume sealed. Same was for the bottom hole, where a bigger hole has been drilled to allow a plastic cylinder of 3.5 mm diameter to function as a hard outlet, regulated by an open-closed valve, to expel struvite accumulated on the bottom. The outlet hole for the feedstock fluid was placed half height of the cylinder and the hole made inside the acrylic wall was sealed by a small diameter hard rubber, and the tightness of this hole was assured by some turn of adhesive tape.

Although the fixed external position of inlet and outlet holes, their real locations were set by small rubber pipes developing their length in the inner volume of the cylinder: the inlet, starting from the top rubber lid, was set to go all along the column and end approximately 5-8 cm before the bottom. The outlet line, starting from its hole at the middle of the column, was instead pointing up and ending at the top of the column, approximately 1-2 cm under the liquid surface at the top (assuming the volume completely filled).

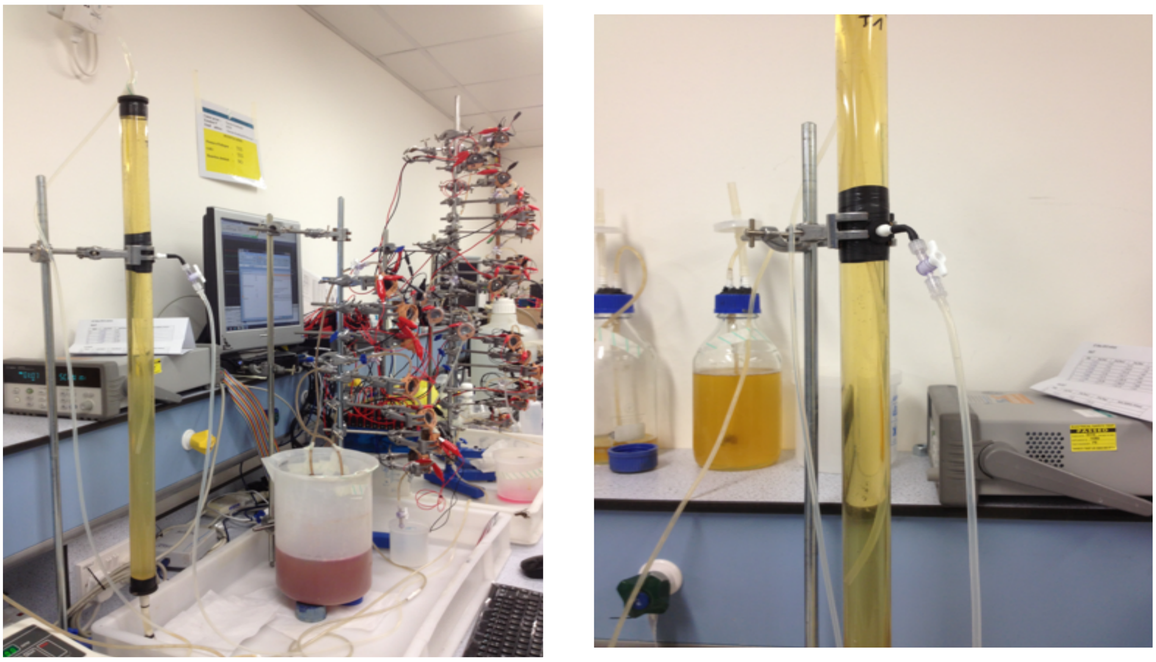


Figure 3.11 - Settling column for struvite collection with high flow rates.

This design has been very effective in accumulating struvite sands travelling along the initial part of the tubing; crystals entering the column from the bottom were directed by the inner tube downward. Tending to follow their own motion, once they exit the narrow inlet tube, the bigger volume suddenly encountered, was leading them to slow down and finally, their weight to prevail among the motion forces, causing the particles to precipitate on the bottom. Furthermore, the direction of the vertical fluid climbing up, along with the regular geometry of the pipe, would allow mostly laminar flow, working favourably to the precipitation of the particles.

A third solution purposed, was adopting a different working principle, aiming at not to draw any debris from the main tank, more than trapping it somewhere along the system.

Its very simple design, made by a plate of thick styrofoam (approx. 3 cm thickness), with a surface area of 10 x 10 cm in this case, was pierced in three points in a row to allow the pipe to enter from the upper surface, be blocked in the desired position, and exit facing the bottom. Four plastic bars, length 4 cm, were inserted partially (1 cm) inside the styrofoam lower face, with the aim of being a sustain for the plate and the pipe. The main working principle within this design was to allow the inlet to draw always from the surface level of the urine, where the sediments are less likely to be presents, and to stop it above the layer of sediments, once the urine within the tank was about to run out.

An alternative design for this model, involve the usage of a same styrofoam plate, but with bigger surface area, almost the same diameter as the internal diameter of the container (generally a regular Becker for chemistry labs) to increase the stability and prevent the whole floating system to tilt.

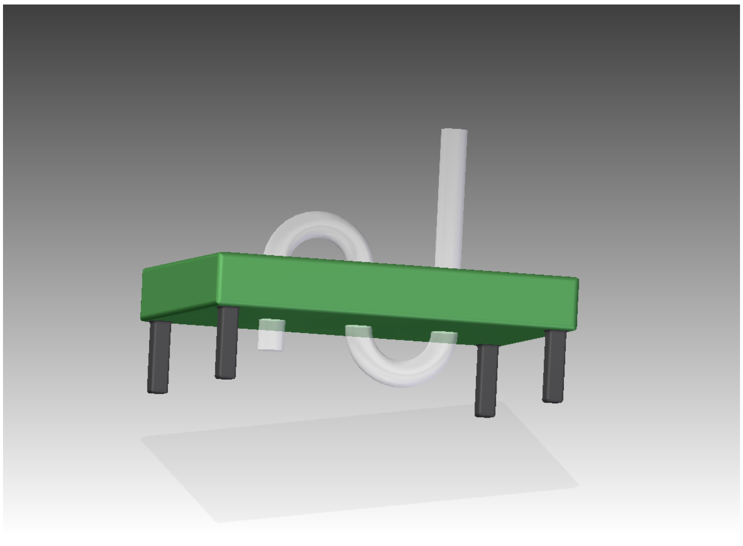


Figure 3.12 - Drawing of third solution: float that hold the inlet tube close to surface and far from the bottom of the container.

Samples analysis

After having struvite collected as wet solution with urine, the two were separated by filtering the solution with thick filter paper. The struvite remaining on paper was than dried by normal ambience temperature, leaving only powder or mica-like crystals.

Samples of struvite were collected mainly from the source bottle, where struvite was accumulating mostly. Also every time a slow COD (within the flow rate experiment) was performed and the stack emptied, a lot of sediments were coming out from anode volumes constituting other possible samples.

After having it dried and collected into vials, samples were better crushed with a chemistry mortar. The resulting powder placed on apposite supports with small circular sticky surface. Samples were than ready for EDX-ESEM X-ray microscope.

With specific software it has been possible to observe each sample prepared, to have reading of which chemical compounds were contained and also to take pictures of characteristic crystal shapes. For each sample it has been made an average of the composition among three readings done.

3.2.3 Results

Samples analysis gave the confirmation that precipitations occurring within the system were effectively struvite. Also the typical shape of crystals has been found many times with the microscope.

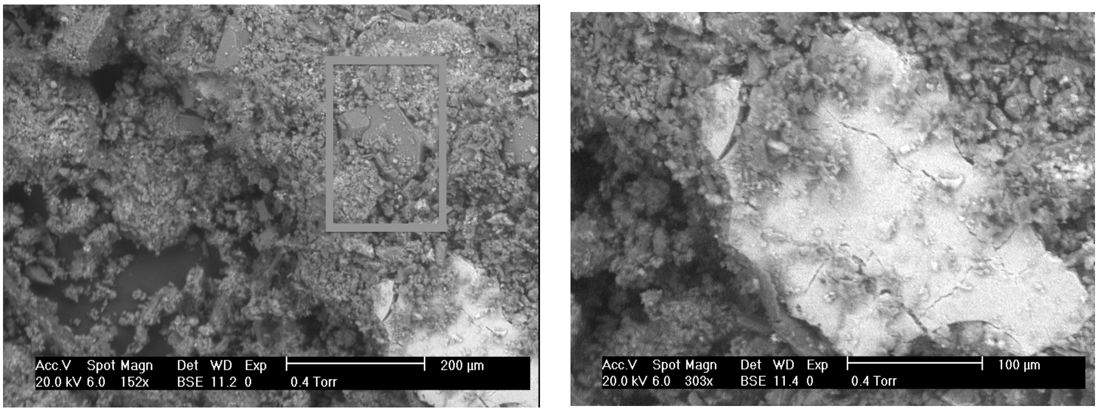


Figure 3.13 - Struvite crystals at the electronic microscope.

Independently from the source, and despite the fact of an external different colouration of few samples (the ones coming from the inside of anode chambers were more brownish than the others), the average composition was always matching.

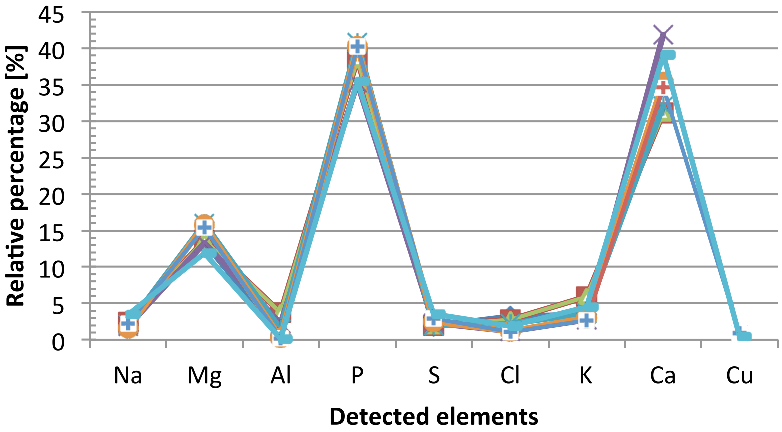
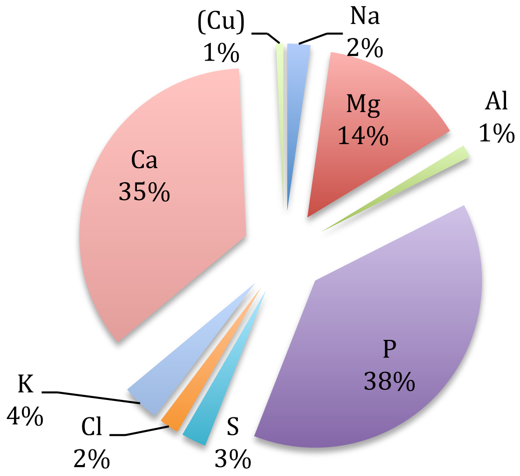


Figure 3.14 - Various readings of struvite precipitations obtained with ESEM-EDX analysis

The relative percentage of values reported, express the absence of *C* and *O*, as for this type of microscope is impossible to distinguish oxygen from the sample from oxygen from the above atmospheric air. Carbon and Oxygen readings from EDX-ESEM are not taken into account.

Average composition has been calculated and reported below.



Overall samples average composition:	Na	Mg	Al	P	S	Cl	K	Ca	(Cu)
	2.26%	14.8%	1.23%	38.68%	2.43%	1.93%	3.70%	35.57%	0.71%

Figure 3.15 - Average composition of struvite collected

Confirmed the fact that struvite precipitations were occurring within a MFC stack, was now taken into account how effectively were the different designs of settlers purposed.

First design with rectangular bottle was not very efficient, because its small volume was not allowing turbulent flow generated by incoming urine stream to slow down becoming laminar. Turbulent motion was causing mixing of the urine, resulting in a general cloudiness of the internal volume. Many struvite particles have been found inside the outlet pipe of this bottle, so the design was finally dismissed.

The settling column tried after, has shown instead to be very effective in trapping particles originated from the urine bottle. Its big volume developed mostly in vertical length was correctly allowing the presence of a laminar flow and letting the particles precipitate; a decreasing cloudiness was observed from the bottom to the top, where the outlet was positioned.

This settler only negative aspect was its high internal volume, which was entailing a high hydraulic retention time at modest flow rates. This characteristic has to be considered undesired every time the quality of the urine was measured along the stack, as a long time was required to fill the empty settler before the “fresh” urine could enter the stack, affecting greatly urine aging and therefore analysis such as COD measurements.

An opposite evaluation could be done instead for fast flow rates (4 rpm, 8 rpm) as HRT of the column decrease drastically and also such device become even more required due to the elevated amount of debris drawn by the flow outside the tank-bottle.

Analysing the effectiveness of settling device tested so far, has to be underlined the fact that their position after the pump was not protecting the latter from the problem of blockages. Piping within the pump has to be considered very delicate, because its continuous being stretched or pressed was favourable to the formation of clogging and struvite big agglomerates, making the pump one of the weakness points of the feeding system.

A solution could effectively being found by placing the settling column right after the urine tank and before the pump inlet, working therefore under negative pressure instead of positive.

Also the third solution analysed within this chapter took into account the importance of preventing clogging within pump tubing. In fact its aim was not to trap the debris anywhere but instead to avoid sucking them.

This floating inlet has been tested with positive results within an experiment requiring flow rate at 8 rpm (approx. 2200 ml/day). Despite the fact of having proved a big amount of debris at fast flow rate, the possibility of set the height of supports allowed to keep the inlet as far as wanted from the struvite layer depositing on the bottom of the tank.

Any possible problems within the usage of this solution could for example derive from unwanted tilting of the styrofoam resulting in exposing the inlet to air and not sucking anymore feed. Solution to this problem have been positively tested within other stack experiments, by adopting bigger surfaces of floating plate which would interfere with tank wall an keep the horizontal position.

3.2.4 Conclusions

A trial of contrasting the problem of debris clogging MFCs stacks has been developed within this chapter, not without positive results and effective solutions. Anyway, the guarantee of avoiding clogging and system blockages is still hard to be reached with this design of fuel cells.

The reason has to be mostly found in their small anode inlet an outlet, in the presence of anode carbon veil mostly occupying a great volume, all together not promoting any ejection of material entering the anode volume. It has been shown within other studies, that different designs of MFC with cylindrical anode chambers and bigger in/out have been more effective.

The problem connected with accumulation of struvite within anode chambers has been widely experienced with the “flow rate experiment”, at high flow rate, even causing difficulties to clear the flow.

The usage of settlers to filter the incoming flows may not be enough if the purpose is to guarantee absence of blockages: other struvite will generate within the internal volumes of the stack, mainly because pH increments.

Once more time, the design and the willing of not to interfere biofilm conditions within the duration of an experiment, makes it difficult to get rid of struvite sands accumulating.

The problem of debris accumulated inside anode chambers wasn’t engaged within this study, but analysing the overall situation of MFCs, and also referring to experiment of flow rate, it is impossible not to take into account how the presence of a thick layer of debris inside the anode could possibly decrease the power of absorption of organic compounds by the biofilm, due to decrement of active surface offered to the flow.

Pictures below show the big amount of struvite extracted from approx. 65 cm^3, volume of the overall main stack.

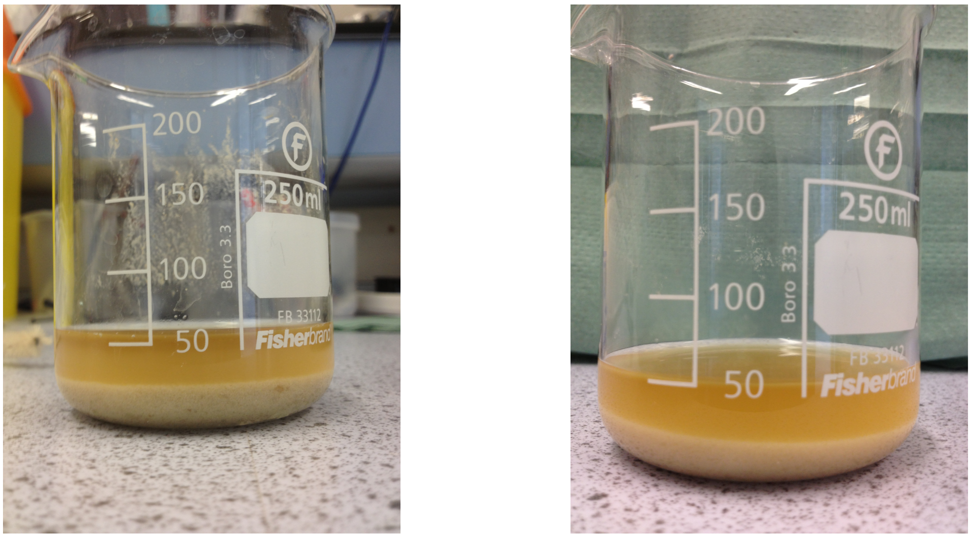


Figure 3.16 - Struvite collected from main stack with a week of high flow rate feeding.

The picture on the left shows the amount of struvite sands expelled from 18 MFCs after one week flow at 4 rpm, before performing a *slow COD*, the picture on the left, refer the same volume, after only a week of flow at 8 rpm. These amounts have to be considered remarkable if compared with initial stack empty volume.

Struvite as a valuable subproduct

Finally, it is worthy to consider one of the minor reasons why struvite problem has been taken into account: the possibility of obtaining another valuable sub-product from an operative MFCs stack processing urine.

Nowadays, there are already companies basing their whole business on extracting struvite as fertilizer for agriculture. Addressing this cases, it is possible to say, through the knowledge developed during this study, that a combination of struvite production and electricity production is absolutely possible.

It is correct that struvite mostly generate when the pH gets alkaline, disadvantageous condition for the power production, which give its best with fresh urine (usually acid pH between 4.5-6). And so, the developing of conditions for struvite precipitation is inversely proportional to the power production, but it is also noticed that still an intermediate amount of energy is produced as long as the feedstock is provided. So power production could in this case become a secondary product, but still something valuable. It should be considered the example of a flow, already set up for other purpose: the main and solely consumption of a MFC stack, the pump power absorption, become in that case null.

Other methods available to separate struvite from urine, such as cooling the urine itself, will accelerate the process of precipitation from solution, still without altering the pH, how has been shown by other experiments.

Chapter 4 Experiments over MFC stack

4.1 Optimal flow rate analysis (Stack)

4.1.1 Introduction

With microbial fuel cell, constancy with power production has been assured by continuous flow feeding system, which delivers, without interruption, fresh urine to the bacterial colony at the anode.

Precedent studies have been showing the advantages of continuous flow compared to batch mode feeding; based on that, this section purpose a further investigation of the continuous flow best operative conditions, by analysing some of the variables involved.

In order to make MFCs a useful application, a considerable amount of energy has to be produced; so far this has been possible only by stacking various units, making them working together as a bigger fuel cells team (anodes and therefore bacterial communities were connected).

With the purpose of doing so, the first important parameter to control exactly is how much fresh feedstock get consumed step by step, and know for example when it has reached its maximum consumption to be useless for further units.

Is in similar cases useful to increase the flow rate and drive through the system more organic matter to allow optimal feeding to all MFCs? The understanding of such aspects could possibly lead to a higher power production, justifying the interest in flow rate investigation.

It is in fact the aim of this study to investigate the relationship between flow rate and power production, in order to discover if there is an optimal condition able to give a best power production or on the contrary if the flow rate isn’t a key parameter within MFCs.

From another point of view, analysing the trend of MFCs power output at different flow rates, will also allow to set the limit of a maximum feeding speed, as the flow rates that will start to separate biofilm particles, possibly damaging the biofilm by removing an amount of bacteria able to deliver electrons.

To try to fully understand the phenomenon of organic matter consumption and its optimum condition within a microbial fuel cells stack, both the "electrical side" and the biological side have been investigated at different increasing flow rates.

Polarizations have been performed to measure the effects of different flow rates over the power production. At the same time, COD analyses were performed to measure the organic matter consumption and associate it to the relative power output.

The data from polarizations and CODs at stable conditions were collected along an operative period of almost two months, increasing the flow rate from a low one of 6.012 ml/h (or 144 ml/day) corresponding to 0.5 rpm, up to 94.65 ml/h (2274 ml/day) corresponding to 8 rpm.

Results highlighted were different from ones expected of an ideal Gaussian distribution among the flow rates, but still a consistent pattern resulted: an optimal flow rate has been individuated as the slowest one, agreeing with higher hydraulic retention times. On the side of COD consumptions, also interesting evaluation could be done from this experiment, concerning the higher consumption obtainable by an increasing number of MFCs, but also about the effectiveness of the method utilized, as the more adequate to derive organic matter real consumptions.

4.1.2 Materials and Methods

The experiment has been conducted with the main stack, composed by 18 MFCs divided into 6 triplets, with hydraulic and electric connections as explained extensively within the first chapter.

During the whole duration of the experiment, a constant set of resistors has been applied to the triplets, in order to exclude one of the variables. The resistance applied, were decided through values given by the most two recent polarizations, performed right before the beginning of the experiment and therefore reflecting the real power condition of the stack.

Those resistances set, called initial set, are summarized below:

Chart 4.1 - Load set applied to main stack during Flow Rate experiment

Triplet n°	**1**	**2**	**3**	**4**	**5**	**6**
Resistance [Ω]	300	300	300	500	1000	1000

The experiment was structured in five different stages, each one repeating the same procedure at a different flow rate.

Measurements of the flow rate were done preliminarily, as described within the appendix Pump Flow Rate on pag. X. Flow rates were tested in increasing order, following the list:

Chart 4.2 - Flow rates tested during F.R. experiment

[RPM]	**0.5**	**1**	**2**	**4**	**8**
[ml/h]	6.41	11.052	20.09	45.4	94.75
[ml/day]	153.8	265.3	482.2	1089.5	2274.1

The starting point of this sequence was chosen due to *Watson & Marlow* pump minimum speed: 0.5 rpm. Starting from the slowest speed and increasing gradually, would allow the stack to adapt with more ease and give more regular and stable behaviour, optimal to perform tests and measurements.

Each stage involved an initial phase of power output stabilization, followed by a sequence of polarizations – stabilization - COD analysis – stabilization, all repeated at least three times to obtain average values.

An ideal stage would be consisting in 48-72 hours of initial adjustment to the new flow rate: fresh urine is supplied on a daily basis, same for manual hydration.

The first test to be performed is COD “A Fast mode”: the evening before the test, fresh urine is loaded into the system, and hydration is applied around 3 hours before the beginning of COD sampling. Stable conditions which would allow the COD to be reliable, were verified through a regular and as much constant as possible power output. Fast COD was allowing a real time view over carbon compounds consumptions[2] and it was not disrupting the general stability of power output, allowing to fast proceeding to the following test.

Assuming that the power output was stable for more than 12 hours, Polarization “A” was following: loads were disconnected from each triplet the evening before, and fresh urine was loaded too. Time overnight was more than enough to reach flat open circuit voltage by the following morning. The following day hydration was supplied and polarization was started about 2-3 hours later.

Once completed, the initial resistance set was reloaded on each triplet; among the 12-24 hours were than left to the system to regain steadily the equilibrium. COD “B Slow mode” was the analysis performed subsequently: fresh urine was loaded and manual hydration was given, to preserve the optimal condition of power output. On the following morning again hydration was applied and after having seen voltage stabilizing, system was emptied completely and carefully, as explained inside the procedures section. Fresh urine was pumped at the inlet of the system and test was started with fresh urine entering the main stack. It is worthy to recall how this kind of COD sampling is ideally the more correct to perform with the purposes of measuring how a same portion of substrate get consumed by subsequent passages through the stack.

After having performed a slow COD, 24 hours were left to the system to readapt from the lack of substrate. Polarization “B” was next on the schedule: same methodology of loading fresh urine and hydrating each MFC, same unloading the triplets the evening before the test. Polarization performed and initial loads reconnected immediately.

At this point of each stage, generally a further third COD & Polarization “C” was run to double-check either a slow or a fast COD, or also a mismatching MPT value.

A single flow rate stage would last generally between a week and two, also depending on unwanted flow blockages. Once the stage was satisfactorily completed, it was possible to increase the flow rate throw setting the pump at higher rpm, and proceed with another stage.

It has to be considered that if polarization tests were requiring 2.5 hours, independently from the flow rate speed, schedule for COD Slow experiment and intervals required for stabilization, could vary a lot between early and late flow rates

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[2] *View Fast and Slow COD procedures within Appendix.*

tested. It has been measured in fact a very different HRT for the main stage subjected to different flow rates:

Chart 4.3 - Hydraulic Retention Times of main stack, at different flow rates

	0.5 rpm	**1 rpm**	**2 rpm**	**4 rpm**	**8 rpm**	**16 rpm**	
$\dot{V}$ [cm^3/s]	0.001779	0.003067	0.005581	0.012611	0.026233	0.053511	
HRT [min]	606.0	351.4	193.1	85.5	41.1	20.1	**All main stack**
HRT [h]	10.1	5.9	3.2	1.4	0.7	0.3	
							Triplet
HRT [min]	93.6	54.3	29.8	13.2	6.3	3.1	**1**
	102.0	59.2	32.5	14.4	6.9	3.4	**2**
	78.3	45.4	24.9	11.0	5.3	2.6	**3**
	79.5	46.1	25.3	11.2	5.4	2.6	**4**
	79.4	46.1	25.3	11.2	5.4	2.6	**5**
	80.8	46.9	25.8	11.4	5.5	2.7	**6**

Time required for Slow COD at 0.5 rpm is 10.1 hours, excluding the time for collecting each of the seven samples that were taken without accelerating the flow. Time instead for 8-rpm stage was of only 20 minutes. It is easy to understand differences: how stabilization times among each test should be long (around 12-24 hours) for slow flows, and instead could be short (around 3-5 hours) for fast flow rates such as 4 rpm or 8 rpm.

Even if stability and regularity concerning power output was a priority for the experiment so to be able to respect the planning, it was very plausible the occurring of blockages within the system, or loss of fluid from any junctions, or running out of urine during weekends and all these were causing a big decrease within power output and subsequently delay with the planned tests.

The general plan adopted for this experiment is summarized below:

Chart 4.4 - Planning of Flow Rate experiment

Flow Rate				
0.5 rpm	**1 rpm**	**2 rpm**	**4 rpm**	**8 rpm**
Stabilization	Stabilization	Stabilization	Stabilization	Stabilization
COD A slow	COD A fast	COD A fast	COD A fast	COD A fast
Polariz. A	COD B slow	Polariz. A	Polariz. A	Polariz. A
COD B fast	COD C fast	COD B slow	COD B slow	COD B slow
Polariz. B	Polariz. A	Polariz. B	Polariz. B	Polariz. B
Polariz. C	COD D fast	COD C fast	COD C fast	COD C fast
	Polariz. B	Polariz C	Polariz C	Polariz C
	COD E fast			
	Polariz. C			
	COD F fast			

To be noticed is the absence of a third COD analysis at 0.5 rpm, because at the first stage the necessity of a third evaluation wasn't been considered yet. Second stage, 1 rpm, had an historical of few subsequent blockages, and some of the initial COD tests were highly affected by unstable stack conditions.

4.1.3 Results

Power output

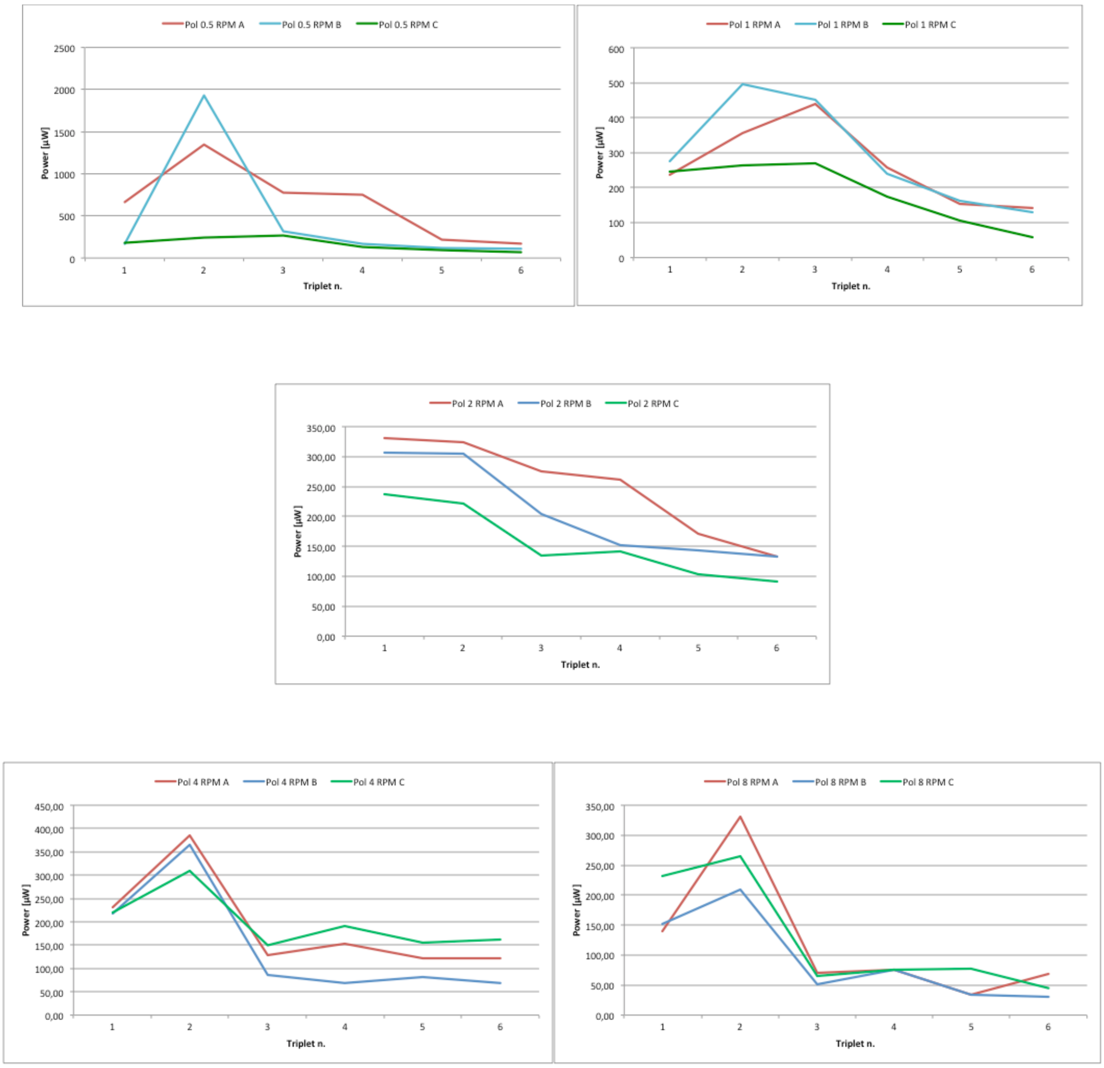


Figure 4.1 - All polarizations performed within Flow Rate Experiment. Following order: a) 0.5 rpm, b) 1 rpm, c) 2 rpm, d) 4 rpm, e) 8 rpm.

Results from each polarization performed are represented above with the intent of showing when the stack was keeping a constant power profile, or if the stack undertook big power output variations.

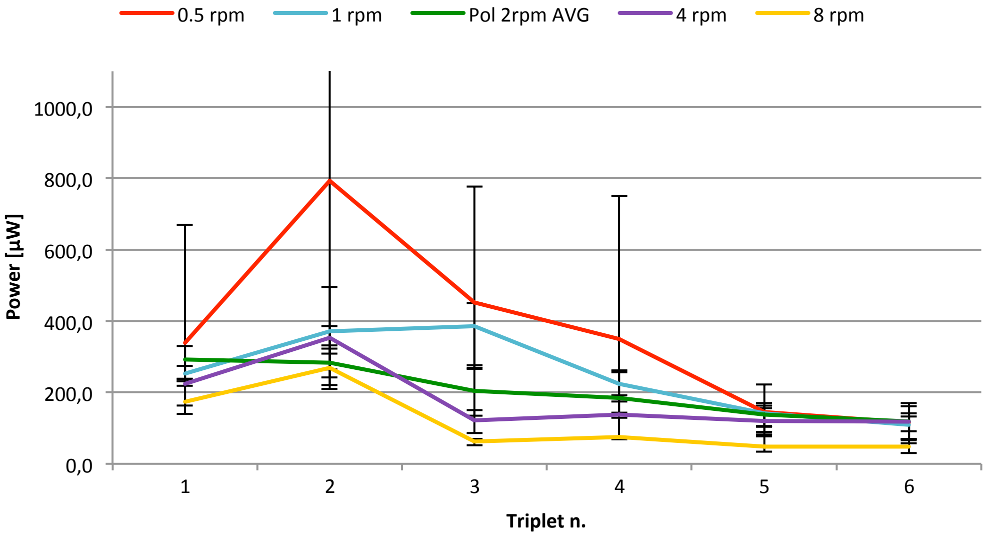


Figure 4.2 - MPT averages for each Flow Rate tested, with error bars.

Averages calculated from Figure 4.1 are represented on the summary view above (Figure 4.2), reporting error bars. Those points, linked by lines, have to be considered as the averages, single MPT values of each triplet. It isn't yet a cumulative analysis.

It has to be taken into account the possibility that during *Pol. A 0.5*, the connectors on Triplet 2 were showing a false contact, keeping the triplet at an overblown higher voltage.

Despite this (even considering minimum error bar shown for triplet 2), it is possible to observe that a regular pattern was presenting, with higher power at slower flow rates. Except for the last two triplets, power output at 0.5 rpm is distinguishing higher than others.

This graph might also show a constant weakness of triplet numbers 5 and 6, last on the chain of feeding. Independently from the speed the feedstock was provided, the power recorded was constantly low. Such malfunctioning condition has been treated with all the possible options during this research, with poor results. Former researchers working with the same group of MFC were already noticing extreme weakness of those elements, therefore it could not only be a matter of not receiving enough food, but more a weakness or scarcity within the biofilm. It might be a good idea to re-establish a new bacterial biofilm within those MFCs. This will be matched also with the information about COD reduction of this triplet.

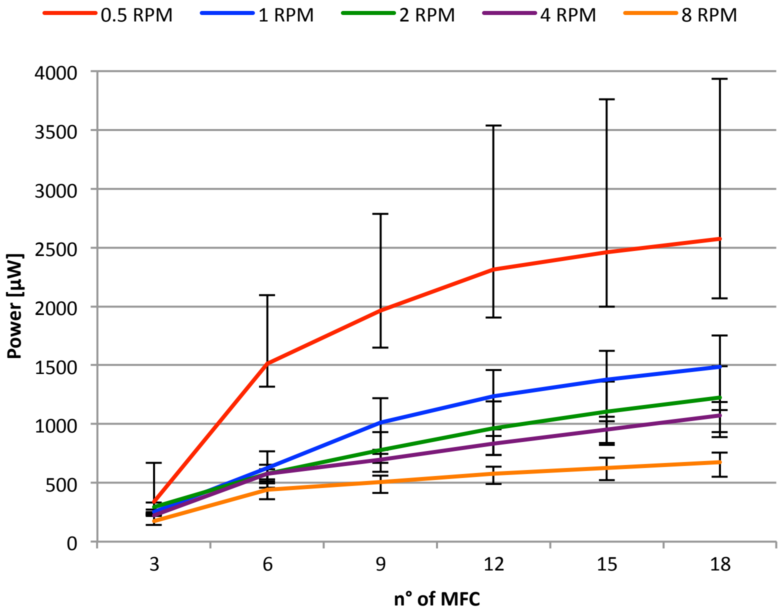


Figure 4.3 - Cumulative average power output at different flow rate.

Displayed on the graphs above, are the cumulative polarization power outputs, average values completed with error bars.

Especially Figure 4.3 summarize a key result: it is showing how effective is an increasing number of fuel cells in the stack, over the total power produced, at each different flow rate. How much advantage could be obtained by adding other units, for each different flow rate, underlining this last, as a primary variable of the study.

Figure 4.3 represent the cumulative power output of each polarization performed, Figure 4.2 the averages of the same. It shows a neat gap between 0.5 rpm power output and all the rest; even if contribution of T2-0.5rpm could be distort, minimum error bars shown that 0.5 rpm was anyway constantly performing better.

Evaluating so far which flow rate is most suitable for a maximum power production, definitely the pattern here highlighted would say the slowest: 0.5 rpm (and 1 rpm) are definitely producing more energy.

This could be explained analysing different aspect:

1. A slow flow rate is allowing a higher HRT, with the direct consequence of giving more time to consume the organic matter available. More time to consume the feedstock would logically result in more electrons released by

the biofilm, as a result more power. This logic could confirm the decreasing trend obtained: the lowest power is gotten with the fastest flow rate.
Within a logic of stack, in which MFC were working as a "dis-assembly" line for the carbon compounds, it is possible to assume that not all the compounds in urine were ready for an immediate absorption, but instead the majority of it was needing some preliminary passages before reaching the best condition to be absorbed by the biofilm. All this was done by the first MFC on the line, making the compound ready for following units. The phenomena could occur at its maximum rate only if the flow rate was slow and giving enough time; this hypothesis is supported by the higher power output obtained at high HRT. Big consumption within the feedstock may lead to the assumption that no more organic matter would be available for the final triplets; this will be dealt with the next figure.

2. Power output has been decreasing by increasing the flow rate, considering between the first and last flow rate tested a big time duration. This decrement might be explained with the accumulation of debris (struvite crystals) within the anode chamber. This phenomenon, time by time was creating a thicker layer over the bacteria biofilm, obstructing an optimal surface exposition to the flow, and decreasing the ability of absorbing nutrients. As this struvite sand could form agglomerates with fluid pressure, it could really become a shield to urine contact with biofilm. As shown in the struvite chapter, faster flow rates caused transport of struvite particles within the anodes: almost one third of internal volume has been found occupied by struvite, all of it within the anode volumes (Figure 3.16).

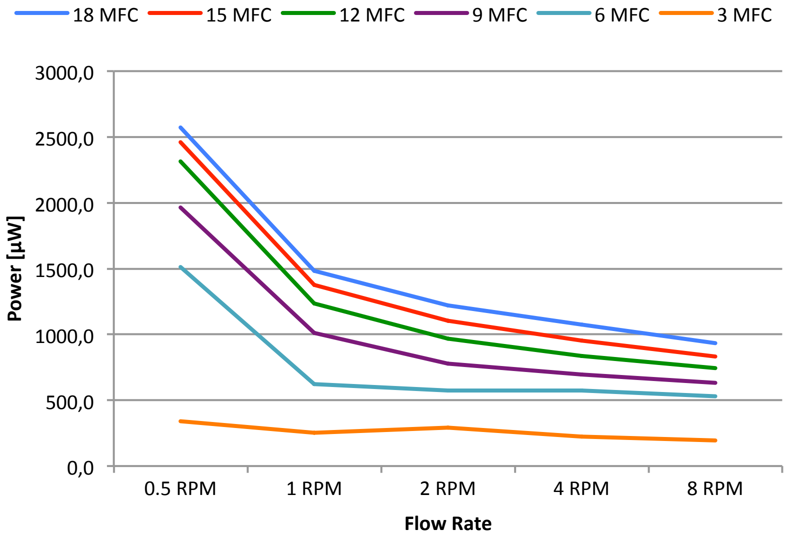


Figure 4.4 - Power variations for different kind of MFC stacks, at different flow rates.

Figure 4.4 is the second key result in terms of power analysis within this study. It shows how much power could vary by varying the feedstock flow rate. It also does that by taking into account stacks of different dimensions: from 3 MFC up to 18 MFC. Influence of flow rate over different dimension of stacks is than shown in this graph too.

It is possible to deduce that:

1. As stated at the beginning, the biggest power has been obtained at slow flow rates, speaking about this design of MFCs.
2. At slow flow rates there is a big difference between the contribution given to overall power output by the first triplet or by the last. This could be extrapolated by reading vertically the different gaps between the starting points of each line (vertical of 0.5 rpm e.g.). At 1 and 2 rpm, so heading towards right, this characteristic is still visible but it is fast disappearing, as arriving at the fastest flow rates. At the fastest flow rate in fact, power increase approximately by the same amount every triplet added to the stack (always reading vertically); the increment is also minor than the one at slower flow rate. This would confirm the theory of "dis-assembly" teamwork of MFCs, in regard to carbon chains, favoured at slow flow rate.
3. It is possible to confirm [12] how effective could be the addition of more unit at the same stack (logic of stacking), and at the same time that has been anyway encountered a limit with the units added. At slow flow rate, it is justified a smaller number of fuel cells, as big increments are obtained till 12

MFC in stack, and than 15 and 18 MFCs don't give bigger increments. On the contrary for fast flow rates, it seems more justified to keep adding MFCs to the stack: it is possible to assume that the trend of increment of power will continue by adding further units, due to a margin of increment dictated by the power of slow flow rates.

With Figure 4.5 below, the attention is brought on resistance values associated to MPT obtained with polarizations during this experiment. In other words, these loads are the ones allowing the maximum power production.
Each values obtained, refer to one of the six triplets, at a specific flow rates. These values are strictly connected with internal resistance R_{int}, very important parameter within MFC that allows to really understanding the health condition and the goodness of the power delivered.
The best condition is obtained where the R_{int} is as low as possible: high value of external resistance required at MPT is than a sign of a bad R_{int} and generally of not optimal condition of the MFC/triplet.

$$R_{int} = \frac{V_{OC}}{I_{load}} - R_{load}$$

Therefore the equation shows how with high external loads, there will be a low current (I_{load}) making the first term at the right side big and therefore big the R_{int}.

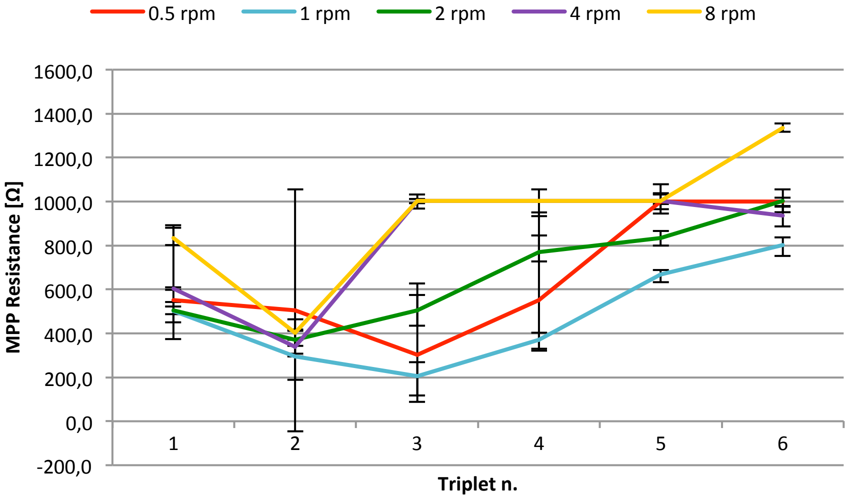


Figure 4.5 - Average of resistance obtained from polarizations, for each triplet.

According to Figure 4.3, with Figure 4.5 it is highlighted an increase in R_{int} by increasing the flow rate, displaying from another point of view the worsening performance with the increasing flow rates.

Higher internal resistance means difficulty in the electron delivery, bad conductivity, hence here it is the possible link with the accumulation of debris within the anode chambers, concurring in lowering the performances of the main stack, that will be discussed later.

Carbon compounds consumption

COD decrements have been measured over all the different flow rates of the experiment and average readings are represented with figure Figure 4.6 below.

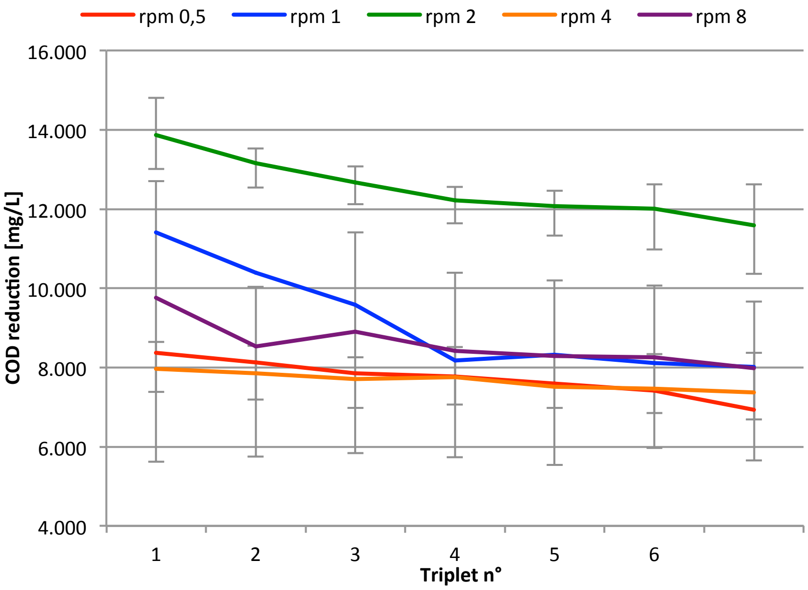


Figure 4.6 - COD reductions measured and initial COD readings.

With Figure 4.6 and the error bars represented, it is wanted to show the high variance of the readings that will entail to not consider absolute the value represented (even if they are already average values).

Following, the individual COD reduction of each triplet has been calculated as difference between COD at the inlet of the triplet and at the outlet; Figure 4.7 shows those reductions.

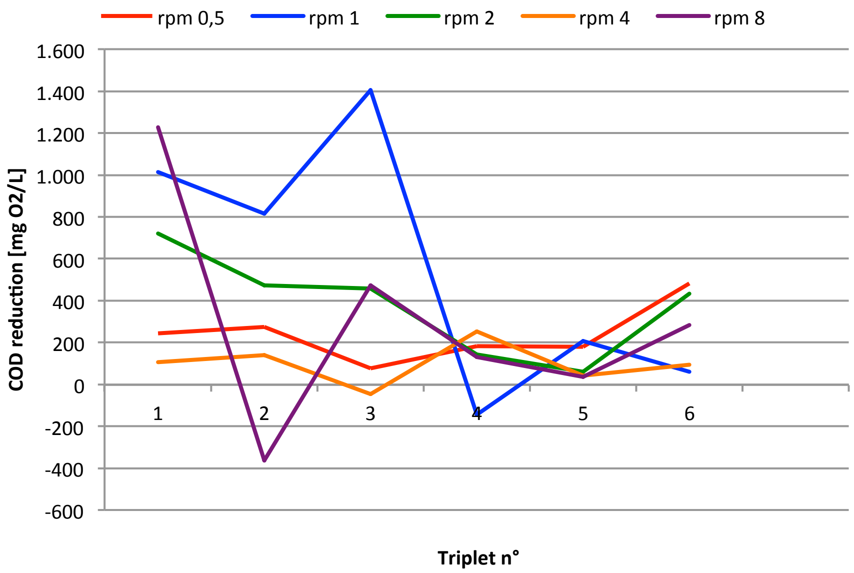


Figure 4.7 - COD reduced by each single triplet at different flow rates.

Chart 4.5 - COD reduction for each single triplet.

	[mgC/l]					
	Tri 1	**Tri 2**	**Tri 3**	**Tri 4**	**Tri 5**	**Tri 6**
rpm 0,5	243	273	77	183	180	483
rpm 1	1015	815	1405	-145	208	60
rpm 2	720	473	457	143	60	433
rpm 4	106,25	140	-46,25	252,5	42,5	93,75
rpm 8	1227	-363	473	130	35	283

In order to show how the pattern is irregular and how very different COD reductions had been measured for the same triplet, it is possible to refer to exact amounts of Carbon unit reduced, from Chart 4.5.

The main reduction occurred always within the first three triplets, denoting mostly healthiness of the MFCs. Considering for example triplet 1, high COD red. is obtained at 1 and 8 rpm, than the lowest at 4, etc., this want to be once more demonstration of an irregular pattern.

Almost no reduction has been recorded for triplet 4 and 5, with a final, constant increment at all flow rates, for triplet 6.

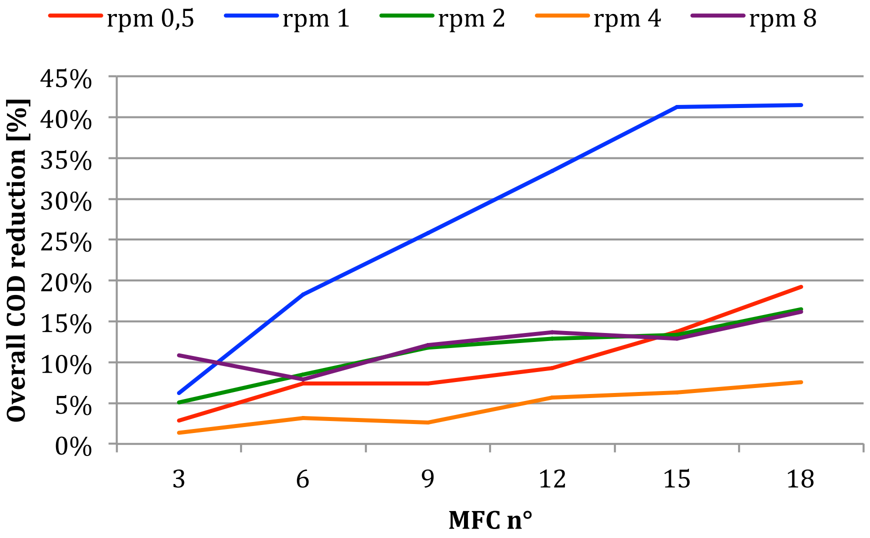


Figure 4.8 - Cumulative COD reduction by adding units, expressed as percentages.

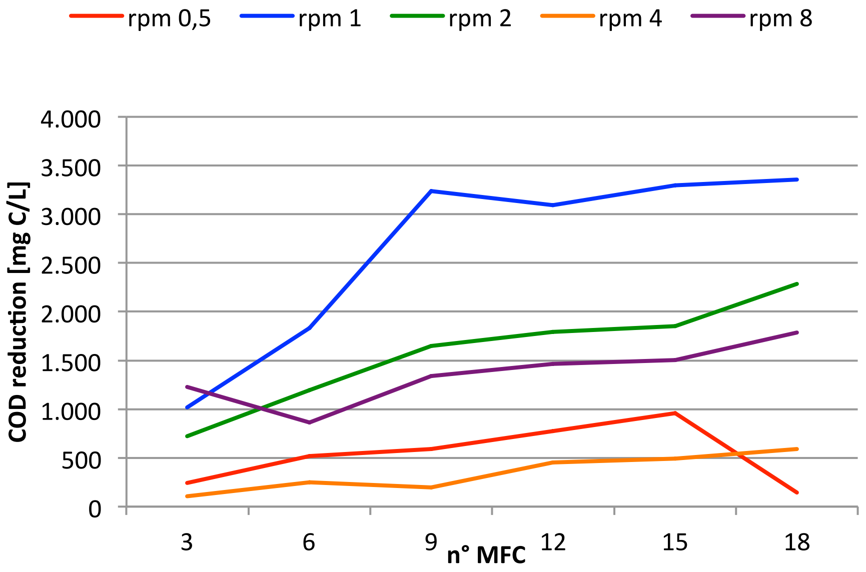


Figure 4.9 - Cumulative COD reduction by adding units, expressed as mg C reduced.

Figure 4.9 represent the cumulative curves of COD reduced at different flow rates. According to common usage, it is expressed as percentage. Also an equivalent graph is reported within Figure 4.10, expressing COD as absolute units of carbon reduced; in the opinion of the researcher this way is more accurate and meaningful than the simple percentage, as neither the initial COD content of fresh urine, nor the triplets reduction was ever a constant, making therefore percentages difficult to be compared.

As for power output, also COD reduction has been evaluated in relation of the number of MFCs connected into stack. The aim, equally, was to understand how carbon consumption would change if more units are added. The values here represented are all averages of multiple readings for each flow rate.

By looking at Chart 4.6 below (mind exact COD reduction within Chart 4.5) it is possible to understand:

1. There is a single case in which COD reduction has been extremely high, reaching a > 40% final value, equivalent to 3500 mg C/l reduced, that was for 1 rpm flow rate.
2. At 1 rpm, the whole reduction occurred within the first 9 MFCs, staying subsequently constant. These data proof that to obtain such a consistent reduction could be used fewer units than the 18 available.
3. There is no regular increasing of COD reduction with the HRTs: the maximum reduction is at 1 rpm, than 2 rpm, 8 rpm, 0.5 rpm and 4 rpm. The expected behaviour of the stack would be in fact bigger reduction at slow flow rates, where there is more time to consume the substrate (high HRTs). This should lead to considerations about effectiveness of this method to measure carbon consumptions related to power production as, instead, a regular pattern was shown for the power vs. flow rate.
4. As explained in literature [8], at high HRT, also the oxygen penetrating the membrane will contribute to the oxidation of organic compounds. It has than to be expected that slow flow rate reductions were even enhanced by O_2 oxidation. COD did anyway not confirm this for the 0.5 rpm.

Chart 4.6 - COD reduction, cumulative percentage of carbon consumed.

	n° MFC					
	3	**6**	**9**	**12**	**15**	**18**
rpm 0,5	2,86%	7,38%	7,39%	9,28%	13,72%	19,24%
rpm 1	6,20%	18,28%	25,84%	33,41%	41,24%	41,46%
rpm 2	5,08%	8,46%	11,77%	12,86%	13,35%	16,47%
rpm 4	1,36%	3,13%	2,61%	5,69%	6,30%	7,53%
rpm 8	10,83%	7,90%	12,10%	13,69%	12,92%	16,15%

By analysing the same data of the chart above, not by "increment of MFC" but by "increment of flow rate", it is possible to represent Figure 4.10 below.

This is also a key-graph concerning the results from COD analysis:

1. Lines that touch among themselves, or equally corners almost one over the others, means about no reduction made by a triplet, even if the same was producing power. This is theoretically impossible, considering that whenever power was produced carbon must have been consumed.
2. Since the lines of increasing number of MFCs are in the correct increasing order this time, it is proofed that increasing the number of MFC will increment the total reduction. It is shown a peak at 1 rpm, and also that the last 3 MFCs don't give any contribution in that case.

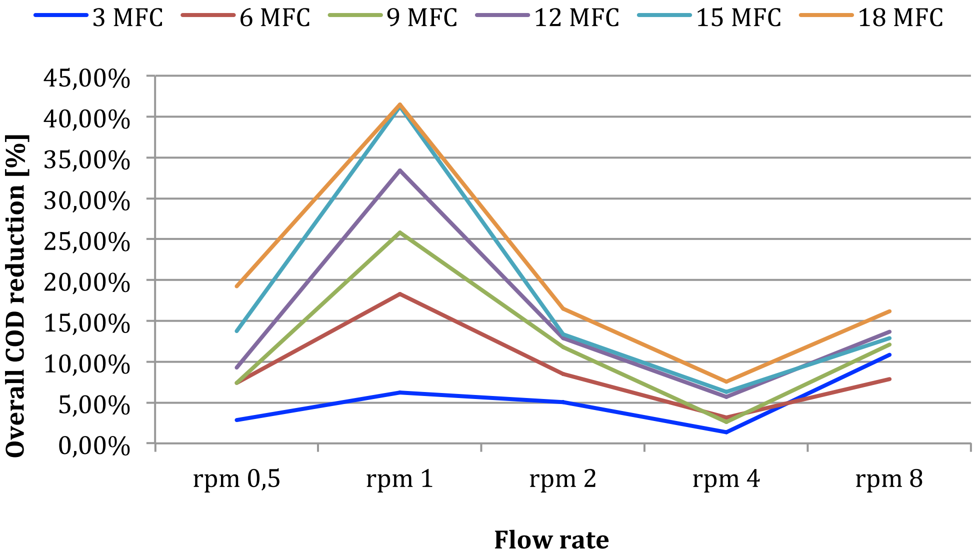


Figure 4.10 – Cumulative COD reduction at different Flow rates, with stack of different sizes.

4.1.4 Conclusions

The results of this experiment shown the highest power output obtained at the slowest flow rate.

A peak of 2300 µW was reached at 0.5 rpm with only 12 MFC on 18, with minor contribution from the following MFCs (a total of ~2550 µW). This could possibly confirm the hypothesis of teamwork when breaking down carbon compounds, actuated by MFCs when connected in hydraulic series.

A fast decrement of power was recorded by increasing the flow rate, according with the decreased HRT. Analysing this aspect it was taken into account the problem of debris, possibly responsible for decreasing the active surface of the biofilm: at the end of the experiment, with 8 rpm, the total power output was only around 900 µW.

The highest COD reduction, of 3500 mg C/l (40% of the initial carbon) was obtained at 1 rpm. COD at different flow rates shown a distribution curve regularly increasing till 1 rpm, and than decreasing. A slight increment is recorded again at 8 rpm: it could be attributed to cleared anodes, again finding confirmation about theory of debris interfering with biofilm activity. Another possible explanation could be found considering 8 rpm flow already in the turbulent field, for this diameter of tubing and anodes; this will involve particles settling into anode volumes to be dragged within the flow, partially solving within the stream carbon compounds previously settling.

Unfortunately the substrate fed within this experiment was, for its nature variable, and wasn't possible to have a constant initial value. Also due to this reason, the behaviour of COD reduction was not constant, and it wasn't possible to directly correlate Power production with COD reduction.

Nevertheless, merging Power and COD analysis, better performances among all the flow rate tested were obtained around 0.5 and 1 rpm, here locating the optimal operative condition, searched as main target of the experiment.

Further observation of the data highlighted that at 1 rpm, the COD initial value was around 12000 units, an high value that confirm the theory of higher reduction at higher initial COD contents. Following, within *COD vs. Load* experiment, it will be shown a strong relation between initial COD and COD reduced.

Based on analysis carried on so far, it has to be questioned the effectiveness of COD measuring method in order to quantify consumed carbon with urine: has been the volatile COD measuring the most appropriate? Despite a very big attention with the procedures, sampling and stack good condition, many time were obtained too fluctuating readings, or negative consumptions, or no consumptions, which was impossible to accept, as a power output was being produced.

4.2 Correlation between COD consumption and Power Output (Triplet)

4.2.1 Introduction

The aim of this section was to understand how much the organic compounds of urine got consumed, with the same fuel cells, under different loads, therefore different powers.

By knowing that carbon compounds are the solely source of energy inside urine, it was a target to verify if there was correspondence in consumption of organic matter, going from a not optimal load, to a more optimal load.

In order to conduct this experiment, a group of three fuel cells, electrically connected together in parallel, with MPL cathode, anti-dehydration lids and well performing stable conditions was used.

Feedstock was provided through hydraulic connections between anode chambers and was delivered at the pump speed of 1 RPM (11.04 ml/h).

A variable resistance, same as one utilized for manual polarizations, has been chosen to provide loads, thanks to the high range of resistance obtainable. A series of decreasing loads from 10 000 Ω to 10 Ω was decided to be tested; within this list of resistance, Maximum Power Transfer resistance was included and known from previous polarizations.

Every stage of the experiment was corresponding to a different resistance. Three different samplings taken every stage, where COD and stable power were measured, at the same load condition, on subsequent days, when fresh urine was supplied from less than 24h. After completing a stage, load was changed and measurements repeated not before having reached again power stability.

Expected results would have shown a higher COD consumed at loads closer to the optimal MPT load, due to increasing power production. What has been obtained instead, didn’t proof the initial thesis, but shown instead a correspondence in terms of power efficiency, and also a very big influence of initial carbon content of the fresh substrate over COD reduced, almost expressing a linear dependence.

Conclusions will than explain why this experiment led to consider direct COD measurement itself, not an effective method to measure carbon consumption occurred on relation of power produced by a MFC.

4.2.2 Materials and Methods

Three MPL-cathode microbial fuel cells (n° 23, 24, 25) were composing the triplet used for COD measurements within this experiment.

MPL was left by choice on these MFCs, that didn’t had their cathode substituted with AC, to match with other experiment requirements, however, these fuel cells have shown along weeks of recorded power output both a very good output and an ability to maintain the power output constant. They were so considered a good test bench for this experiment.

MPL MFCs were adopting close-to-air cathode for improved hydration stability.

MFCs were connected electrically in parallel and than feed in series, so COD reduction operated by the first will sum to the second and so on for the third, resulting in the triplet total COD reduction. Microbial fuel cells were disposed as vertical column and feedstock was supplied from top to bottom, following gravity.

Before starting the experiment, two polarization of MPL triplet, were performed, in order to know MPT and optimal load; this was resulting in 1000 Ω and average MPT of 85 µW (87 µW on Polarization 05/07 and 83 µW on Polarization 12/07).

The series of resistances to be tested was:

Chart 4.7 - Series of load applied during the experiment.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
10000 Ω	5000 Ω	2500 Ω	1000 Ω	500 Ω	100 Ω	50 Ω

This was decided based on MPT knowledge, with the aim of testing both very high (10k Ω) and very low (50 Ω) resistances. A variable resistance was so connected to the triplet contacts.

Flow rate was set to 1 RPM (11.04 ml/h) because it has been proved from “flow rate experiment” to be the best in terms of power output, and also, because it was convenient to adopt a “slow” flow rate that will allow enough hydraulic retention time and give sufficient carbon reduction to be clearly measured.

Experiment started from the value of 10k Ω, fresh urine was added and MFCs hydrated on a daily base, to provide maximum carbon availability and best operative conditions. Triplet was let on normal operative flow to allow fresh urine to go through all the volumes and power output to stabilize. Between 12 and 15 hour after fresh urine addition, a sample of 2 ml was taken from the outlet of the triplet (outlet of the last three anode chambers on the column, the bottom one). The inlet sample was taken subsequently, to not introduce any kind of flow disruption or turbulence due to the opening of the tap. Power output at the time of sampling was recorded and later related to COD reduction.

Inlet and outlet urine sample were than processed with both High Range and Medium Range COD analysis, to have two different values of the same reading to be compared, ensuring that no small reduction was missing due to sampling errors. Medium Range COD was processed with 1/10 dilution rate of urine with deionized water.

Procedure was repeated other two times, at intervals of approximately 12 hours, always assuming very stable power production.

The load was than moved and the sampling procedure was repeated for each of the seven different resistances. Three values of COD reduction, along with COD initial value of fresh urine, and average power production were recorded for each stage.

4.2.3 Results

The difference between COD inlet and outlet readings, for each of the resistances tested, has been represented by dots in Figure 4.11. The numbers next to each dot represent the COD value of urine going into the triplet, as the initial content of carbon within the fresh urine (expressed as *mg C/l*). This entity will be defined as Carbon Initial Content (CIC).

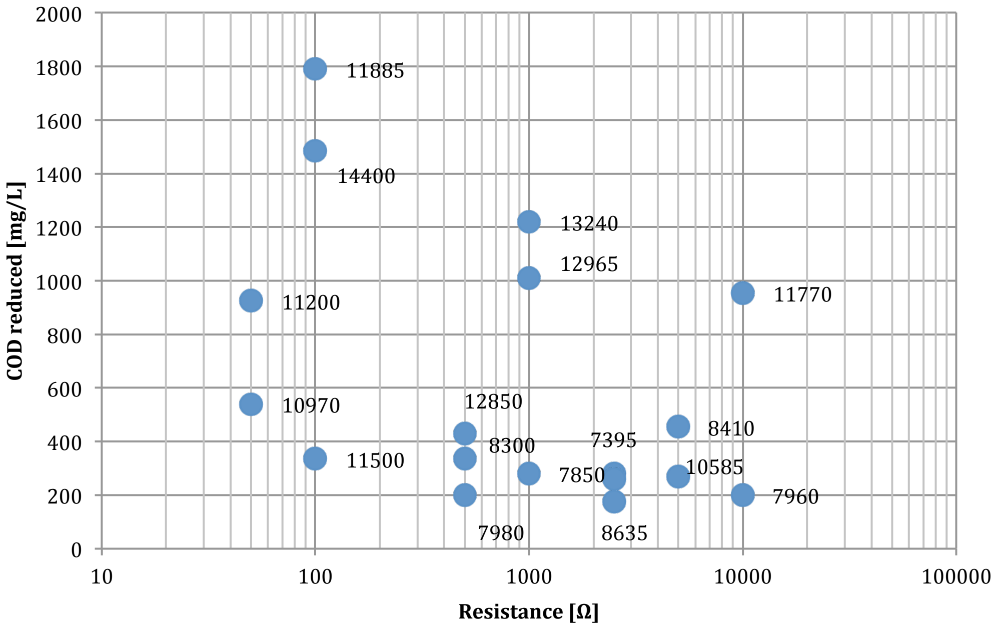


Figure 4.11 - COD reduction at different resistances, with initial COD.

The first result highlighted above is the very different COD reduction verified within the same resistance, almost on every different load tested: from 100 Ω to 10000 Ω, two orders of magnitude more. Measured COD reduction within the same stage of the experiment (same load) could vary from 5 to 8 times (as shown with 100 Ω load).

If loads define the regime of power output from MFC, it is therefore not possible to establish a direct correlation between the COD reduction and the load applied (power obtained).

However, proceeding with the analysis, it is noticeable that high COD reductions occur only when CIC >11000 mgC/l, instead low reductions are more likely verified with CIC around 7000-8000 units (see Figure 4.11). This will be an important deduction, and will be examined in depth following. Just a few readings were out of this trend with low reduction starting from high initial COD values.

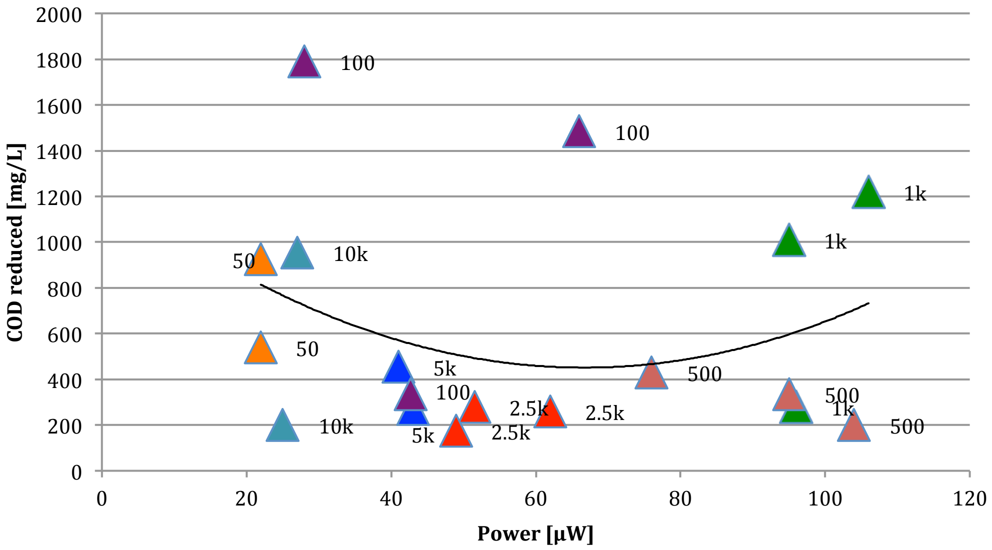


Figure 4.12 - COD reduction related to power production and loads

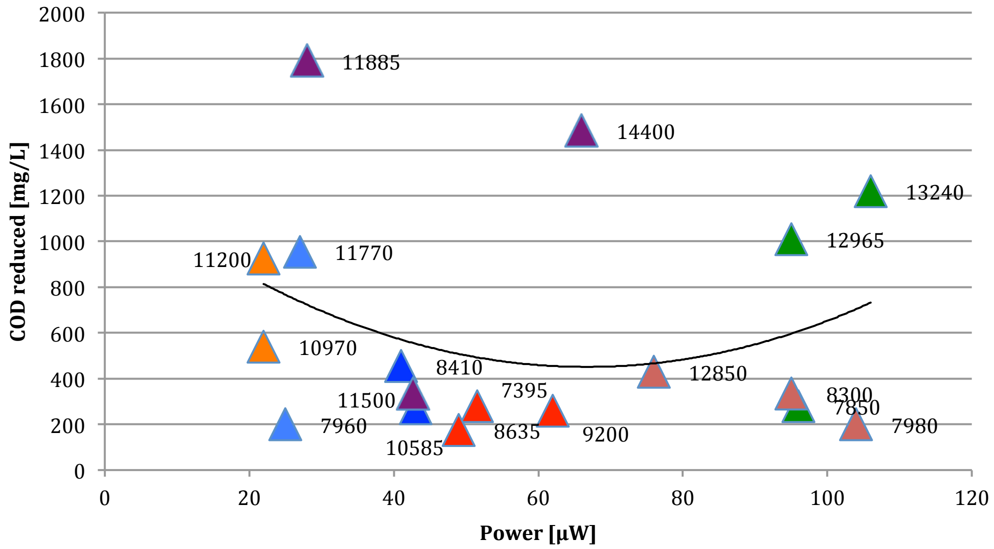


Figure 4.13 - COD reduction related to power and CIC

With Figure 4.12 it has been highlighted that:

a) The highest COD reduction has been obtained at 100 Ω, and that instead optimal load 1k Ω has an intermediate-good reduction, but far from the highest.

b) It was verified that optimal load 1k Ω and values close to it (500 Ω) gives the best power output, so the operative condition shows a well performing MFCs. Since it was matching with initial results from MPT value, it was possible to state that this analysis has been carried on with well performing MFCs.

c) Observing best performances and CIC (carbon initial content) from Figure 4.13 it could be noticed that it isn't necessary a big CIC to obtain a high power output: as shown, the highest power was obtained both from urine with 7500-8000 mg C/l and 13000 mg C/l.

d) In Figure 4.13 moreover, it could be seen that the same COD reduction could be obtained at very different power outputs, as shown with 1k Ω and 10k Ω with a similar reduction of 1000 unit approximately. Second, as noticeable with 500 Ω resistance, the reduction is increasing with higher CIC, but it isn't the power which in that case was decreasing despite the high CIC.

Merging observations from points above it is possible to confirm that a high COD reduction isn't indicating an high power output.

Same statement could be deducted for initial carbon content, as shown at point “c”, again a not compulsory condition for a high MFC performance.

Despite the truthfulness of this last statement, it has to be underlined the fact that initial amount of urea in the fresh substrate was not measured within this study.

Urea constitute around 40-50% of the carbon in urine, but it isn’t utilizable at all by the biofilm in MFCs. Urea is very dependent from pH of urine and tend to increase with it, subsequently decreasing the available carbon to be reduced.

Therefore if an average amount of 50% carbon is considered as urea and discounted from the calculations, the analysis done so far will be still valid, but this aspect could be further investigate with future studies.

These statements are fundamental if we wan to argue about effectiveness of COD as measuring method of consumption within biological fuel cell, in relation with their power production.

The finding at point “c” is important since it has been highlighted along the study that low CIC correspond on average to fresh urine from mixed donors, and it will be the most likely scenario within future application of MFCs for example in public toilets. This point proofs that, if properly set (correct load), mixed donor urine is effectively capable of good power production.

Finally, it could be seen a common pattern, which shows higher COD reduction when the initial energy from the substrate (CIC) is higher, low COD reduction when CIC is lower. This could be seen almost with all the values of resistance tested, especially with 10k Ω, 1k Ω, 500 Ω values and 50 Ω, which clearly represent the whole interval of loads. This aspect will be analysed further with next figures.

MFC Carbon Conversion Factor

To further proceed with the analysis, measured quantities were combined together, and important results came out.

The amount of power produced was divided by the measured COD reduction, this was done for each resistance and results are displayed in Figure 4.14, also including CIC initial values within labels.

$$CCF_{MFC} = \frac{P}{COD\ reduced} \quad \left[\frac{\mu W}{mgC/l}\right]$$

This index, named Carbon Conversion Factor by the researcher, express the ability of an MFC to produce power with a single mg of C available: the higher is the CCF, means the higher is the power produced starting from the same content of carbon.

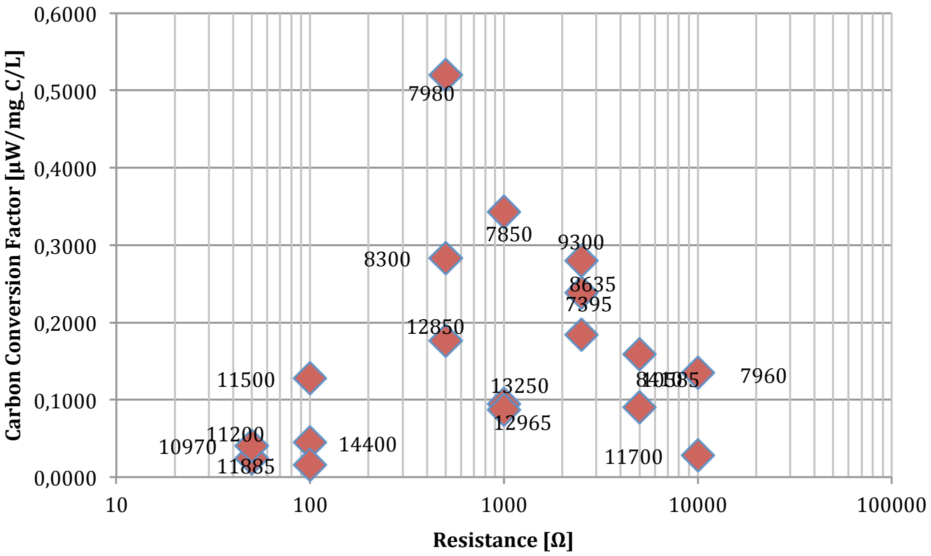


Figure 4.14 - CCF (Carbon Conversion Factor) at different loads, expressing the whole range of functioning condition of a small stack (3 MFC).

Figure 4.14 has to be considered (along with following Figure 4.15) a key analysis of the experiment, showing finally the expected relation about loads and COD: this graph with its neat triangular shape, state that at optimal resistance load (1k Ω) a constant amount of energy available (1 mg C/l) is transformed more efficiently by the MFCs, which are capable of producing more power. Distancing the optimal load, the ability of MFCs to consume the carbon decrease and a smaller amount of energy is obtained both at too high or too low resistance.

Analysing the problem with the CCF mutual value (1/CCF), it is possible to say that this graph proof that it requires a bigger amount of carbon to produce the same amount of energy, at non-optimal load conditions.

What we have seen along the previous graphs (Figure 4.11, Figure 4.12, Figure 4.13) was meant to show that it isn't true that a big absolute reduction generate more power, but here it shows that the power is more efficiently generated at optimal loads, if assuming not to be at saturation conditions.

Observing the values of the best load (1k Ω), it has to be noticed that within the same stage (resistance), different efficiencies were obtained and similar was for others resistances.

It must be taken into account again the CIC: what it shows is that also a lower initial value of COD, concur in giving a better CCF.

High initial COD may give generally high COD reductions (see following paragraph), but if this last is not supported also by a high power production, than the conversion factor here analysed would not be as good.

This Figure 4.14 in fact, shows that MFC triplet never gave high carbon conversion factor when fed with “rich” urine (CIC above 11-12000 units). The reasons for this could be found allowing a saturation condition of the biofilm activity: so far in fact, a lot of initial carbon wasn’t useful to produce more power, but instead, MFCs showed better ability to reduce the carbon when CIC on average levels (7-8000 units), or in other words, when not saturated.

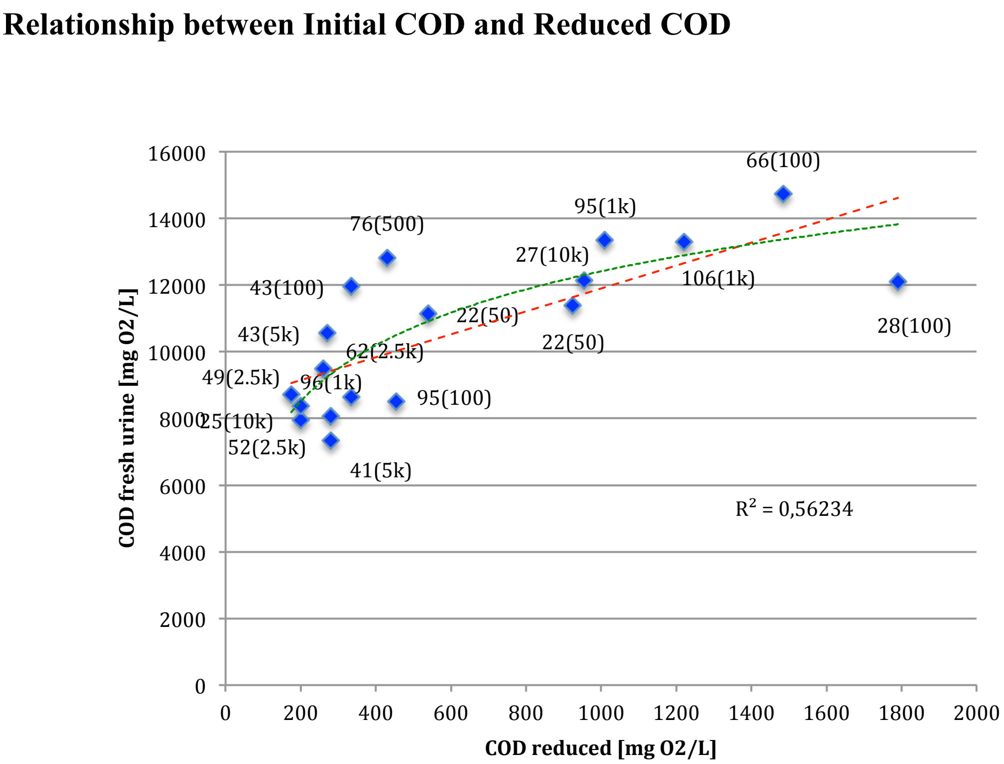


Figure 4.15 - Initial COD content of urine and obtained COD reduction. Within labels, the power produced and the load.

As many time mentioned within the previous analysis, a final relationship has been underlined with Figure 4.15: the COD reduced has been related with its initial COD content (for the fresh substrate).

Two kind of interpolative fitting have been tried: linear and logarithmic; R^2 indices are also reported. Despite the fact that R^2 indices don't show a perfect fit with the experimental data ($R^{2\ \mathrm{Lin}} = 0.56$ or $R^{2\ \mathrm{Log}} = 0.64$), it is undeniable that Figure 4.15 shows an increasing pattern. This could be supported by the big variety of data collected under many different load conditions, constituting a quite homogeneous set.

With this final graph is it therefore possible to say that the reduction of COD is connected with the initial COD content of the substrate, and particularly that higher initial COD give a higher COD reduction. This fact could be a key factor to understand evaluating COD reductions results and dependence factors.

4.2.4 Conclusions

Based on the knowledge that the source of energy for Microbial Fuel Cell is solely the organic carbon, it has to be expected mathematically that this source will be consumed. Particularly, respectable power outputs (such as 100 µW at 500 Ω) produced during this experiment should have been corresponded by high COD reductions.

Despite that, results were not confirming this hypothesis, and so far it is possible to say direct COD measurement cannot be considered as indicator of good or bad MFC performances, as there wasn't such evident correspondence.

Neither it could be done with the initial COD value of fresh urine, at least within this stage of the studies, where percentage of urea was not measured.

With other words, we can't evaluate good functioning of a MFC just based on its COD reduction or COD initial value (as we could do instead with power).

This, as said in the introduction, allowed the researcher to strongly recommend not to adopt the COD as a measuring methods for carbon consumption within MFC.

It hasn't been analysed any other method of measuring the carbon consumed (such as BOD) and this could be a starting point for future research.

If not directly showing any relationship, dependence between COD and power is instead clearly found within the carbon conversion factor (CCF). This study shows in fact that one more passage has to be done to understand connection between carbon consumption and power: at better load condition the MFC works more efficiently, works better. Of course MFC should produce more energy, but also, this same amount of energy, was produced with a smaller amount of carbon, compared to other loads, therefore more efficiently. From another perspective, a same amount of carbon was better administrated at optimal load condition than at others, giving a bigger amount of power.

Here also a matter of saturation of the biofilm was shown, demonstrating that a high content of carbon within the fresh urine, was not a synonym of high power, obtained more efficiently with lower initial carbon contents.

Finally, if still COD want to be used as indicator of consumptions, mandatory is to keep in mind how an high or low initial COD content would influence the value of COD reduced, as proofed within the last section of the experiment.

4.3 COD consumption in Batch Mode (single MFC)

4.3.1 Introduction

A single MFC, with Activated Carbon cathode, acrylic lids over cathode chamber has been utilized within this experiment with the aim of verifying the maximum level of COD reduction if fed with batch mode, obtainable with this specific design.

This experiment locates itself within a context of developing a better knowledge of COD readings over microbial fuel cell consumptions, in addition with the knowledge acquired within the previous chapters.

It has been known from literature that the utilizable carbon source contained inside urine is at maximum 60% (as already said, approximately 40-50% is not-utilizable urea). Anyway investigating COD consumptions of this model of MFCs, continuous flow consumptions showed only a peak of 40% reduction over 100% of the available C, with much lower averages around 20% reduction.

Flow rate experiment, with its COD analysis, showed that with continuous flow the bigger is the HRT, the more is the time available to consume the carbon compounds so bigger should be the COD reduction obtained.

Is it in any way possible to obtain a bigger COD reduction?

Continuous flow and batch mode are very different in their nature, because they bring the biofilm at different working regimes. With this experiment, batch mode was chosen to simulate and measure the maximum carbon reduction obtainable, giving the MFC enough time to consume all that was available within the feedstock.

The experiment was conducted by injecting a fixed amount of fresh urine within MFC22, considered a well functioning fuel cell, due to its very good power output, if compared to other good performing MFC from the main stack. The same urine was also kept in a clean glass bottle, where no reactions were taking places a part from normal degradation due to reaction with oxygen and pH natural changes [8]; this sample of urine was considered a control for all the external agents (different from bacterial).

Fuel cell power output was than monitored and recorded till it was reached a stable unchanged minimum plateaux. At that point COD measurement would reveal the maximum COD consumption obtainable, and the number will be compared with the 60% theoretical value.

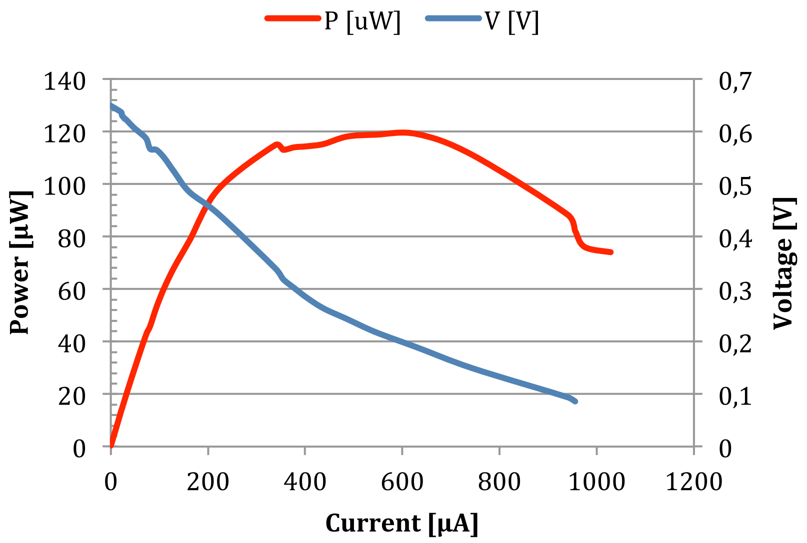
Results will show that reduction obtainable with batch mode by a single fuel cell could reach a maximum of 25% over total (66.7% of the consumable carbon), instead of the 60% theoretical (100% of the consumable).

4.3.2 Materials and Methods

MFC 22 has been chosen to perform *Batch Mode* experiment, due to its good operative condition and high level of power output, if compared with all other units.

The design of MFC 22 is totally equal to the one adopted for the main stack, same design improvements have been applied: installed AC cathode, installed acrylic lids to prevent cathode dehydration.

Polarizations have been performed before starting the experiment, in order to establish MPT load. Below the best and most recent polarization:



MPT [µW]	119.07
R [Ω]	300
V [mV]	189
I [µA]	630

Figure 4.16 - MFC 22 polarization and MPT.

An average has been done with this and previous polarizations, resulting in 1000 Ω resistance applied on MFC 22. This value, in fact, was also consistent for comparison with the main stack triplets, as the better performing were loaded with 300 Ω and connected in parallel of three (1000 Ω equivalent resistance).

Approximately 2 cm of transparent tubing was connected at each inlet/outlet of the anode chamber to ensure complete filling of the anode and eventually display the presence of unwanted air; closing valves were also inserted at the end of each rubber tube to allow keeping urine inside the anode. Overall resulting internal volume was 3 ml.

MFC has been kept on continuous flow before the first run of the experiment, in order to start the experiment at the best functioning condition. Each of the three runs of the experiment was performed as follow: MFC 22 was first manually hydrated, than emptied completely and rinsed gently with fresh urine. Fresh urine from the same day was than injected from the bottom of the anode chamber, volume was filled completely keeping the MFC vertical in order to allow all the air to escape from the top outlet. Valves were closed to seal it. Recording of the power output was started.

Also a small glass bottle was filled with the same fresh urine and left closed next to MFC 22, to ensure the same condition of temperature present in the room. This was intended to be a control of the natural degradation of carbon compounds by oxygen, with absence of any MFC bacterial reactions.

Power output was than monitored daily till it was reaching a power production below 5-6 µW (around the 5% of its maximum level). Test running was than considered concluded and anode volume was completely emptied inside a sterile vial; extracted volume was about 2.5-2.7 ml of urine. Power recording was stopped.

Urine extracted and control urine were than processed with COD reagents both MR and HR.

COD reduction obtained by natural aging was than discounted from the reduction measured with MFC 22, resulting in the real reduction caused by bacterial reactions only.

$$real\ COD\ reduction\ [\%] = \frac{Anode\ COD - Control\ COD}{fresh\ COD}$$

This entity was expressed with *mg C/l* , if related with the volume of urine inserted, absolute *mg C* consumed could be obtained.

Area under curve is calculated for the power output, resulting in the total power produced (*µW h).*

By relating

$$\text{CCF} = \frac{\mu Wh\ produced}{mg\ C\ consumed}$$

it is possible to obtain the Carbon Conversion Factor (CCF) for the single MFC (as for the previous chapter), which would express how much energy could be produced by single fuel cell, introducing *1 mg* of carbon.

Batch mode test has been repeated three times with the same procedure.

4.3.3 Results

Each of the three run results and calculations are summarized with the chart below:

Chart 4.8 - Readings obtained from COD analysis and Power measurements with max t measured.

		Trial A	**Trial B**	**Trial C**
Fresh urine COD reading:	[mg_C/L]	9670	7790	11030
Raw MFC reduction:	[mg_C/L]	1950	515	2815
Raw MFC reduction:	[%]	20.17%	6.61%	25.52%
Control reduction:	[mg_C/L]	360	410	575
Corrected MFC reduction:	**[mg_C/L]**	**1590**	**105**	**2240**
Corrected MFC reduction:	[%]	16.4%	1.35%	20.31%
Total amount C consumed:	[mg_C]	4.77	0.315	6.72
Power analysis:				
Energy provided:	**[uW*h]**	**2072.67**	**1042.98**	**1050.32**
media P:	[uW]	27.728	8.45	11.191
tot h:	[h]	74.75	117.558	93.85
Microbial Fuel Cell Carbon Conversion Factor	**[mWh/mg_C]**	**0.435**	**3.311**	**0.156**

Chart 4.9 - Readings obtained from COD analysis and Power measurements with equal t=48 h.

		Trial A	**Trial B**	**Trial C**
Fresh urine COD reading:	[mg_C/L]	9670	7790	11030
Raw MFC reduction:	[mg_C/L]	1950	515	2815
Raw MFC reduction:	[%]	20.17%	6.61%	25.52%
Control reduction:	[mg_C/L]	360	410	575
Corrected MFC reduction:	**[mg_C/L]**	**1590**	**105**	**2240**
Corrected MFC reduction:	[%]	16.4%	1.35%	20.31%
Total amount C consumed:	[mg_C]	4.77	0.315	6.72

Power analysis:				
Energy provided:	**[uW*h]**	**1640.77**	**690.25**	**719.40**
media P:	[uW]	46,365	16,67	14,988
tot h:	[h]	48	48	48,00
Microbial Fuel Cell Carbon Conversion Factor	**[mWh/mg_C]**	**0.344**	**2.191**	**0.107**

Each power output has also been represented within the graph below.

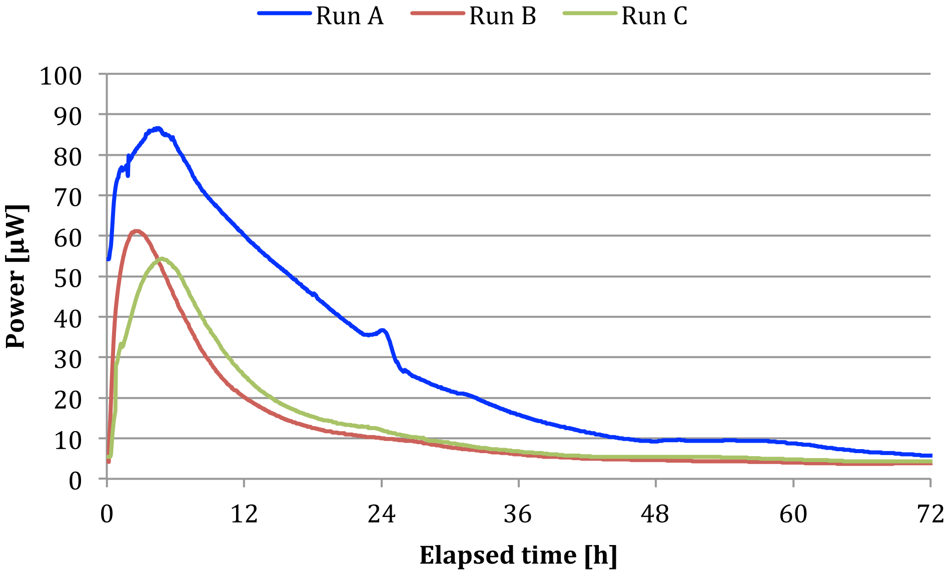


Figure 4.17 - Power output over 48 hours, during each run of COD Batch mode experiment.

First thing to be observed is that on each run the power output reach its minimum level (below 5-10 µW, or <90% of MPT) around 48 hours later, the power output than stays flat indefinitely long, confirming that all the available energy has been totally consumed. Among other things, this fact proofed that the behaviour of the bacterial biofilm was constant, and therefore the MFC well performing, in order to exclude this variable from the analysis.

Aiming at compare power produced over the same amount of time, excluding one more variable, Chart 4.9 resume power output data over 48 hours timespan.

It has to be considered that theoretical optimal COD consumption would have request keeping the MFC on a continuous stirring, to make sure that all the fluid can get in contact with the bacterial substrate; the absence of this feature, may have minor affected the COD consumption, since consumed substrate was occupying the volume directly adjacent to the anode, preventing the still biofilm to reach far fresh substrate.

During the experiment another interesting behaviour of batch mode systems was highlighted:

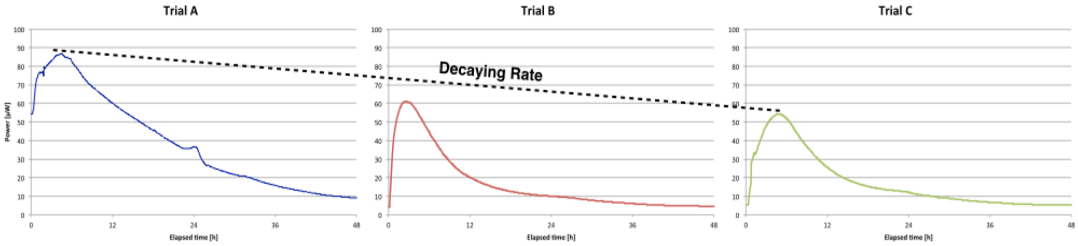


Figure 4.18 - Max power output from MFC in subsequent batch mode runs, showing a clear decaying/mortality factor.

What is shown in Figure 4.18 is a decreasing maximum power output at subsequent runs of batch mode feeding despite the fact of always using fresh urine.

Scientists already know this behaviour and it is called decaying rate, or mortality rate. It shows an increasing loss of activity of the biofilm within the MFC. This is due to the key difference between batch mode and continuous flow: with the first, there is no exchange of consumed biofilm and no waste removal from the anode volume. For this reason the dynamicity of biofilm activity is compromised and optimal conditions for the life of the biofilm are missing, causing a progressive death of the same.

This feature is also the very reason why a continuous flow can't be compared with a batch mode, being intrinsically different the conditions, and being the contact of its same waste with the biofilm a negative factor for its optimal activity.

Moving the analysis on data from Chart 4.9, particularly test B and C, both were giving a comparable power output of 1042 μW and 1050 μW over the 48 hours. Observing the relative "cleaned" COD, reductions recorded were of only 105 mg C/l against 2240 mg C/l: an enormous difference of 95% between the two (COD units reduced by natural oxidation with atmospheric oxygen are of similar entities, so they can be implied.).

These data ultimately combined were also expressing the same discrepancy through the CCF of 3.32 *mWh/mgC* for case B and 0.156 *mWh/mgC*, because of course a same amount of energy produced by a smaller amount of resource, would appear as a greater ability to convert carbon sources.

This once more showed, also with a single MFC, the simplest unit among triplet and stack tested so far, that COD could not be related directly to power as a performance index. Once more the volatile COD measurements used in this dissertation, by itself, could not be considered an indicator of good performances of a MFC or a stack, as instead could the power.

Analysis of the data preceded showing, even with a single unit, that linear relationship between initial and consumed COD (highlighted in the previous *COD vs. Load* exp.) has been respected. It is shown in Figure 4.19 that trial B, following trial A, following trial C have increasing initial COD value and so do the their COD reductions.

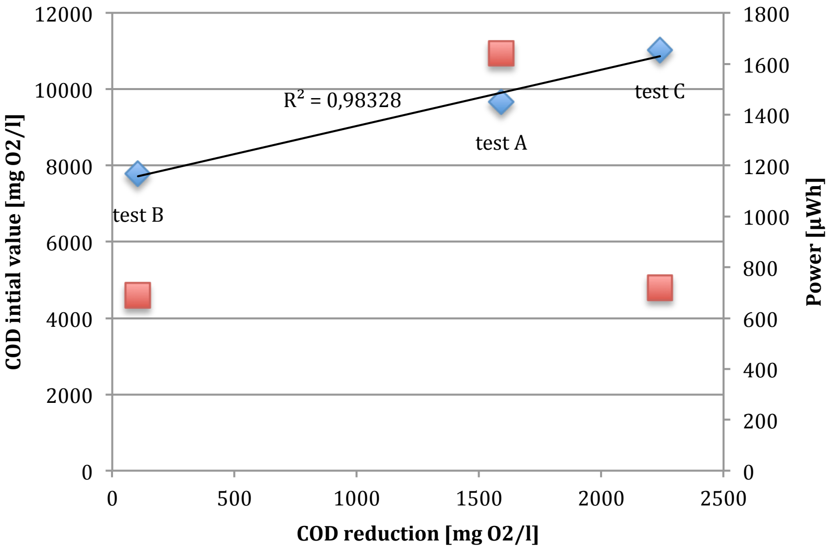


Figure 4.19 - Linear relation between COD reduced and initial value, along with generated power at each COD reduction.

Also total amount of power produced over the 48 h has been represented in the graph above; as it could be deducted it isn't possible to explicit a direct correlation between power produced and COD consumptions, as apparently the biggest amount of energy produced hasn't been generated by the higher reduction (case C), but indeed by an intermediate one (case A). Loads for this experiment were always kept constant.

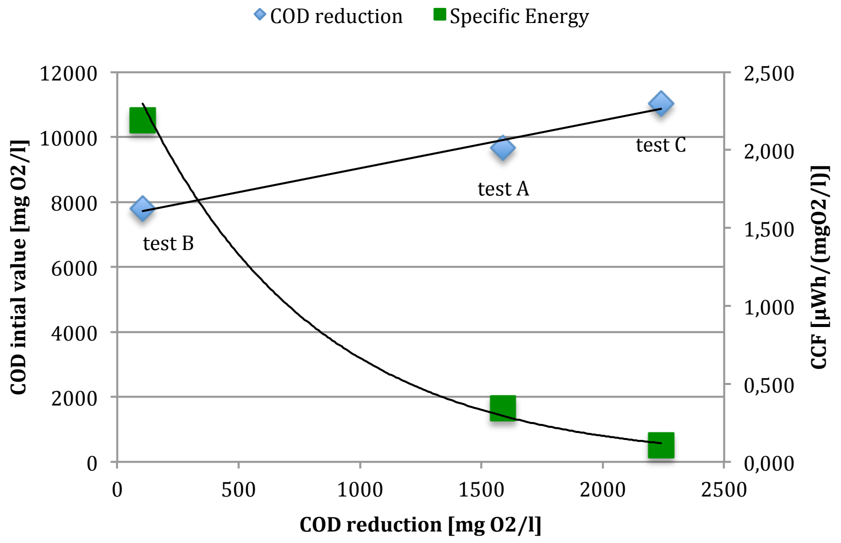


Figure 4.20 - Linear relation between COD reduced and initial value, along with MFC Carbon Conversion Factor at each COD reduction.

Reconnecting the previous analysis of power output and COD consumed it has been again possible to display the same behaviour of power density, as found in the previous chapter with a stack of three MFCs.

Analysing the data from a single MFC, COD initial value once more is very important because Figure 4.20 shows more ability to convert with low carbon content substrate and also low COD reduction, as initially seen in Chart 4.9. Therefore more ability to produce energy with lower carbon content. Since this time, the load didn't change, but the unique variable was the quality of the initial urine and the evaluating output was the power produced, it has to be deducted that a MFC could produce energy more efficiently when the substrate is less charge with carbon matter, probably because it occur a situation of saturation within the biofilm, especially with batch mode, as previously said.

4.3.4 Conclusions

Along this experiment, the ideal COD reduction of 60% (100% of the reducible) of organic matter within urine was never reached.

This experiment could attest a COD reduction of a single MFC in batch mode around 1500-2000 mg C/l reduced, equivalent to 15-20% (~33%), as average of the obtained values.

It is clear from literature that continuous flow COD reduction is under very different conditions respect to batch mode COD reduction.

This intrinsic difference could still be used as a point of force, aiming at verify which behaviours were repeated. Particularly the experiment aimed to give an estimation of a maximum value really achievable by this design of MFC, considering the power produced as an unquestionable indicator of MFC reduction activity.

This experiment on a single unit, due to its simplicity and ability to exclude variables, was a useful bench-test for understand relationship between power produced and COD consumption. Data from COD reduction were so different between each other, even if, instead, power and general behaviour of MFC along time was similar and regular.

Despite 60% is the amount of carbon (excluding urea) utilizable by MFC, it has to considered after this experiment, that maybe that value is only ideal and not practically reachable with these MFCs.

It was anyway interesting to verify that also within batch mode, the relationship between initial COD value and reduced COD value has been respected, here even showing a linear dependence.

Concluding, if analyse the consumptions is the target, than logic would suggest that some data is missing, and COD itself is not a complete and reliable index. This suggestion should be taken into account by future researchers that should try different methods and way to measure the “fuel” consumed by MFC.

Chapter 5 Practical applications

5.1 Self-sustainability: powering of a water pump

The results here reported permit to realize the following scientific publication:
Practical levels of electricity from MFC stacks fed with urine, I. Ieropoulos, P. Ledezma, A. Stinchcombe, G. Scandroglio, C. Melhuish, J. Greenman, 2013, ECS.

5.1.1 Introduction

Powering a real application, with any energy generation device undergoing developments, it's the ultimate sign that improvements and optimizations applied to this last have been successful.

This experiment, even if not the main of this study, has the double intent of verify on a real scale, the improvements made along this project, and also to initiate a pattern of self-sustainability of MFC stack. It is wanted to proof that MFCs combined together with the correct configuration could produce enough energy to provide themselves autonomously of food by powering a small pump.

The triplets utilized for the main stack were now connected together in series, allowing all the voltage to be summed up. A couple of super-capacitors (2.7 V, 5 F) were connected in series, to become the accumulation element of the system.

Once reached the charge required, an electronic switch was allowing the capacitors to discharge their charge and deliver it to a small pump, able to pump a liquid for few seconds.

This experiment was tested twice: at the beginning of the study, with old cathodes (CV) and different hydration, and at the end, after having all the cathodes replaced with more performing ones.

On the first attempt bad health condition of the MFCs, and a too high power-demanding application didn't allow positive results, but on the second attempt the new power condition and the choice of a more power-compatible pump allowed the demonstration to be completed successfully.

With this experiment it was shown that it’s possible to obtain enough power to power a useful application; unfortunately, this test showed also some limit of the actual level of technology, such as long charging time or necessity of resorting to a tricharging process, in which a capacitor is the intermediary and it isn’t possible to connect directly the MFCs to a device yet.

The pattern for new research has been traced by this and similar experiments, the result obtained are encouraging to proceed forward, especially aiming at pairing the MFCs with the low power applications being developed nowadays.

5.1.2 Material and Methods

First attempt

For the first attempt of powering a water pump was used a stack of 12 Microbial Fuel Cell, adopting carbon veil cathode. Those MFCs were grouped by three connected in parallel, resulting in 4 triplets. The triplets were than connected in series one after the other. All of them were free from loads.

The device to be powered was a new design pump, which was requiring 3.5 V to operate.

The accumulation unit requested to deliver such a voltage, was composed by two super-capacitors of 2.7 V and 5 F connected in series, resulting in a overall 5.4V and 2.5 F capacitor. Having smaller Farads, allowed to decrease the R_{int} and obtain fewer losses during the charging process.

Capacitors were connected to the stack and the pump to be powered through an electronic switcher, set to release the electricity within the capacitors once a precise value was reached.

The experiment started from open circuit voltage of 2.36 V and rose in 2 hours at 4.945 V.

Capacitors were than connected and the charging process started: after 4 hours of charging process the voltage delivered by the stack was reaching only 2.48V, with a current from the triplet of 0.1 mA.

Those values were considered way below an acceptable level for well performing MFCs with this design; therefore the experiment was interrupted without reaching the target.

Second attempt

The experiment was repeated after twenty days; within this time, MFC have been subjected to general check up and substitution of obsolete cathode materials. Also 12 fuel cells have been maintained and available since not utilized within other experiments, consequently this time the number of MFC available was increased to 24 (double as before), disposed in 8 triplets.

All the triplets were again connected in series. Also the same capacitors connected in series were used as accumulation units; their overall voltage was 5.4 V and 2.5 F. Again, the electronic board was regulating the charging process, working as a switch between triplets of MFC, capacitors and pump.

For this second attempt, a lower power demanding pump was utilized: mod. M200-S, 3V DC from RS, UK), favouring the work of the capacitors. A small hydraulic circuit has been set up in connection with the pump, where the fluid contained into a transparent plastic bottle was extracted from underneath the surface level and was reinserted from a hole a few centimetres above the liquid surface, allowing a clear view of the on-going circulation, once the pump would have been active.

Experiment started with the connection in series of all the triplets, allowing them to reach a global Open Circuit Voltage: OCV main stack = 4.5 V slightly increasing.

A first demonstration of powering the application was performed by pre-charging the capacitors at 3.28V. This in order to have only the final part of charging process to execute and be able to display the correct functioning of the device connected.

Powering the small pump was accomplished successfully and second recharging cycle started.

This time process started from fully discharged capacitors, from a voltage of 2.4 V and a maximum current available equal to 600 μA. The full charging process allowed also to check the ability of the stack to delivery a constant power and sustain for real a utilizer connected for long time.

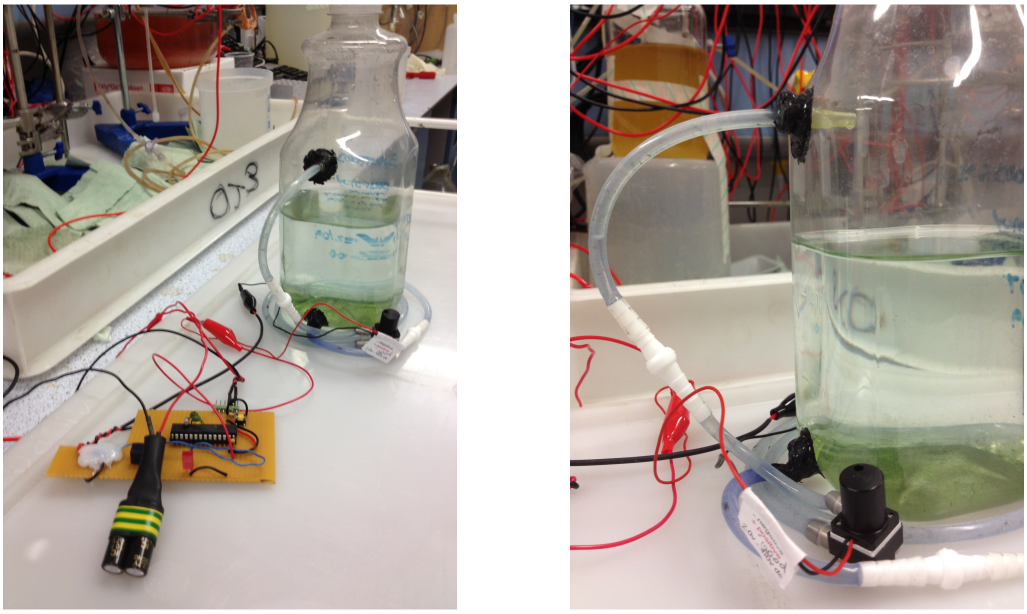


Figure 5.1 - Set up of powering application experiment: 1) two super capacitors, the switch-board, and the pump circuit; 2) detail of the pump and the hydraulic circuit.

5.1.3 Results

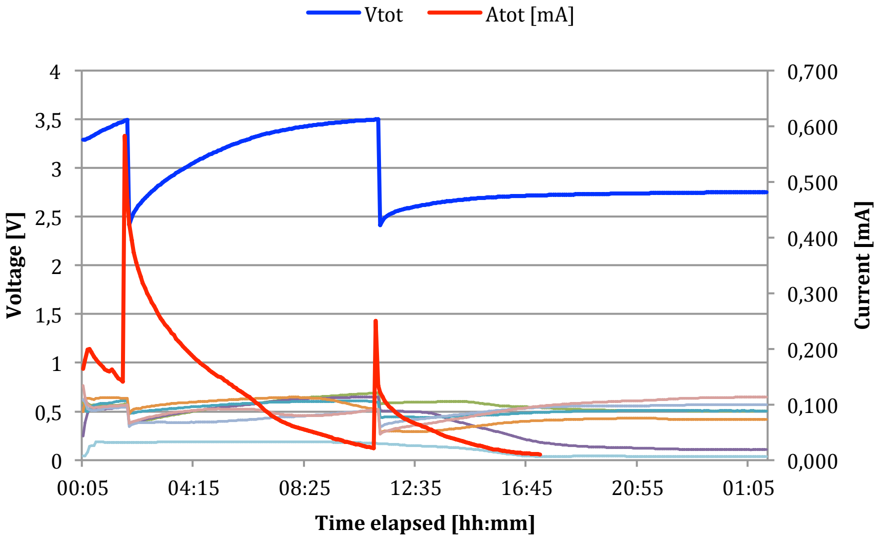


Figure 5.2 - Charging-discharging profile of capacitors utilized within the experiment.

The first powering simulation was performed starting with an initial charge within the super capacitors of 3.28V; the target voltage of 3.5V was reached after 1:45 h, with a minimum current of 140 µA.

Power was than released to the electronic propeller, which was activating liquid circulation.

Discharge process and connected pumping of the flow lasted only around 25-30 s, this is why it isn't visualized within Figure 5.2[3], that has a logging timespan of 5 min.

Powering the small pump was accomplished successfully and second recharging cycle started. This time process started from fully discharged capacitors, from a voltage of 2.4 V and a maximum current available equal to 600 µA.

A full charging cycle for this system require ~9,5 h to complete the charge. On its maximum capacity, the stack delivered an operative power of 490 µW.

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[3] *Current graph has been truncated due to low frequency disturb that was covering the real current output.*

5.1.4 Conclusions

The actuation of a useful device as a fluid-pump was successfully accomplished thanks to the charging process received from the solely MFCs. This is an important demonstration, carried with this small volume MFCs, that powering real application is possible.

Despite the very long charging times that are still occurring, which could nowadays be a limiting factor, nonetheless it has to be compared the failure of the first attempt and the success of the second within a very short time, thanks to occurred technology and material improvements. All this in order to proof how big could be the margin of improvement within this technology, promising great results.

The whole demonstration, more than just moving a device, want to project into future its application, looking at a self-sustainability of the stack, at an integration of different functions within the same robot, solely and completely auto-powered and maintained. A pump for example is a very important device in order to provide MFC with food, and being able to power it by themselves is a great result for a MFC stack. With this and other applications successful powered, the road is surely open.

5.2 MFC, a living sensor: temperature sensing

The results here reported permit to realize following scientific publication:

Chapter 8 – Bodies: Energy and metabolism, by I. Ieropoulos, P. Ledezma, A. W. Xavier, G. Scandroglio, C. Melhuish, J. Greenman.

5.2.1 Introduction

Microbial fuel cells is not only robot powering, not only stacking and not only waste-water treatment. It is a living organism shielded into a case, and, because of its nature, it is really a multitasking entity, able to respond to very different external stimulations with its, yet very simple metabolism and homeostasis.

Integration of different functions, self-reliability and self-control of microbial fuel cells stack are nowadays the key-words for the future of MFCs development, with the relatively new concept of *Living machines* [10].

Imagine about an entire robot made by only MFCs, demanded not only to self sustain itself vital functions, but also to sense light changes, temperature, etc, as an hybrid being that joint the living entity and the mechatronic/artificial hardware for survival and continuous operation of the system as an unique machine [10].

It is indeed the topic of this section to discuss a new possible application for MFCs as *living sensors*: if a biohybrid device can respond with any output to external modifications such as temperature, lights, loads or flow rates, that means they can become controllers for future robots, replacing for example conventional silicon systems which need to relay on electrical input. This could be a very big step for science, confining the traditional electronic only where strictly necessary.

Within this section, the ability of MFC bacterial biofilms to respond at temperature changes will be investigated. It has already been proofed in many studies that the metabolism of bacterial colonies is directly influenced by temperature variations, and that even small variations in temperature, could cause big variations within the bacterial biological activity.

In detail it has been verified a doubling of the reaction rates for a 10°C increased temperature, making MFC behaviour perfectly fitting energy-temperature relation stated in the Arrhenius equation.

Here therefore is an alternative practical demonstration of MFCs not only as powering units, but also as real-word sensors, giving the ability to nowadays scientists to switch traditional silica sensor to a new concept of biological sensors.

5.2.2 Materials and Methods

The experiment of temperature sensing with MFC was conducted with smaller volumes MFCs, if compared to the ones used in the whole rest of the study. Internal volumes of just 1 ml, instead of 6.25 ml; same Nanocure polymer (RCP23 resins doped with ceramic particles) was used to design cathode and anode chambers. Electrodes made by 15 cm^2 of carbon veil were folded and inserted in each chamber. Chambers were divided by cation-exchange membrane (CEM) and gaskets were interposed to ensure water tightness.

Flow rate was provided by a peristaltic pump *Watson & Marlow* at the fast rate of 6 rpm (corresponding to 70 ml/h), and synthetic media (Triptonite – Yeast Extract based). High flow rate was allowing fast adapting of all the components to temperature changes.

Anode side was this time not open to air but subject to a constant hydration process by an equivalent hydraulic system allowing recirculation of tap water, changed on a daily base.

Two stacks of 4 MFCs were utilized for the temperature sensing, both of them equipped with a high precision temperature probe *DrDaq* from PicoLog instruments, installed in the direct nearby of each stack. A third temperature sensor was embedded into the PicoLog board, allowing room temperature sensing.

First group of MFC was left not insulated and open to ambient influence; second group was contained within a 12 cm diameter cardboard cylinder, divided into two halves for accessibility to the inside, and internally covered with aluminium foil to act as thermal insulator for the internal side.

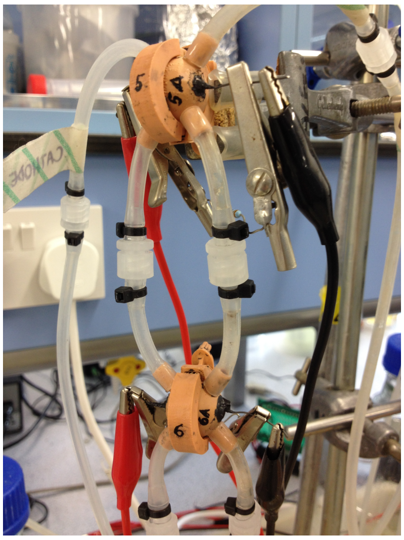

Figure 5.3 - Design of Micro-MFCs (1 ml) used for temperature sensing experiment.

Thermal regulation for the insulated stack was provided by a third hydraulic circuit feed from an adjustable thermic bath, pumping water at constant temperature inside a 3mm diameter rubber tube, all wrap around the second stack, also contained within the insulation cylinder.

An initial calibration for the thermo-regulated stack was performed in order to check effective matching from temperature imposed by the water bath and temperature measured by temperature sensor within stack 2.

Finally resuming, first stack was left to ambience temperature and thus not controllable, second stack was temperature controllable and preliminary test assured the complete insulation from the outside.

In order to analyse the response of a bacterial biofilm to temperature variations, different test were performed, both checking stationary and dynamic response of the system.

Stationary test involved the measurement of power output at steady-state temperatures, by setting the temperature at a precise value and than giving time to the MFCs to reach a steady power output. Measurement was repeated for a series of decreasing temperatures among 43 and 37°C.

Dynamic test was performed instead by slowly increase or decrease of temperature over a long interval of time and measuring the power output coming from stack 2.

A first dynamic run was performed increasing the temperature from 1°C to 26°C over a timespan of 9 hours; a second run instead measured power output over a decreasing variation of temperature, from 43°C to 26°C also along approx. 9 hours measurement.

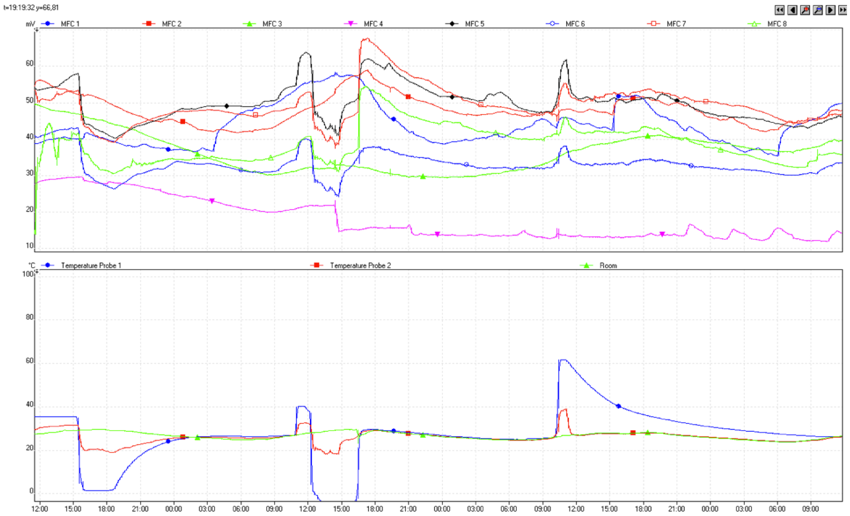

Figure 5.4 - Voltage and Temperature profiles during dynamic temperature sensing experiment.

5.2.3 Results

Smaller volumes of fuel cells were chosen to minimize the thermal latency and response of the system, as bigger volume of fluid would have responded slower to temperature. Temperature variations anyway were applied along considerable intervals (9h), in order not to give thermic shocks to the biofilm but allow its reversible metabolic response.

Stack n°1, not insulated, worked as a control, and by measuring power output variations due to room temperature changes (as seen in Figure 5.4) was ensuring that no influence over Stack 2 was affecting insulated temperature measurements.

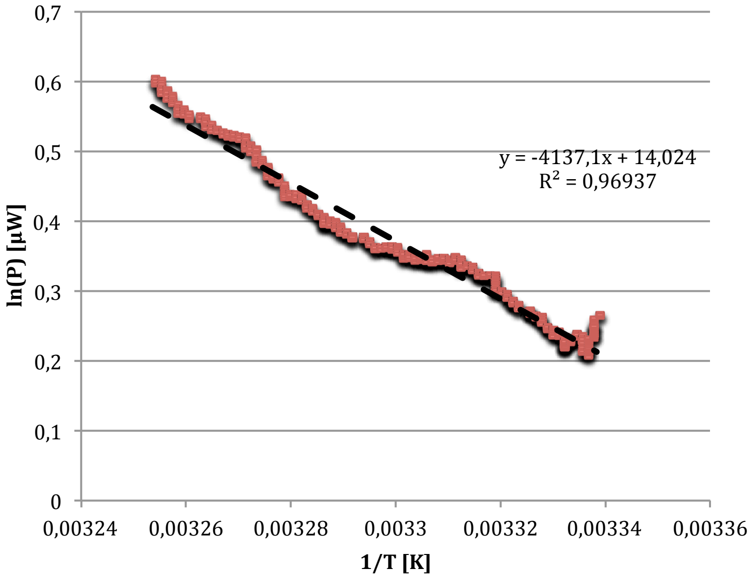

Figure 5.5 - MFC response to temperature dynamic variation over a decrement of 10°C.

Within Figure 5.5 the power output and the temperature relative to dynamic decrement stage (from 35°C to 26°C, descending) are processed to match Arrhenius equation units.

The relationship shown in Figure 5.5 is very clear and precise (R^2 = 0.97): as the MFC decrease in temperature there is an accompanying decrease in power output. Between 25 and 30°C, a ΔT=10°C, there is a 3-fold increase in power, in line with the Arrhenius prediction of a doubling reaction rate (biological metabolism), for an equivalent increment of temperature.

Same behaviour could be repeated, as demonstrating a sort of hysteresis cycle (here not reported due to different intervals of T for the swap up and down), with the temperature and power output increasing, as shown in the following Figure 5.6.

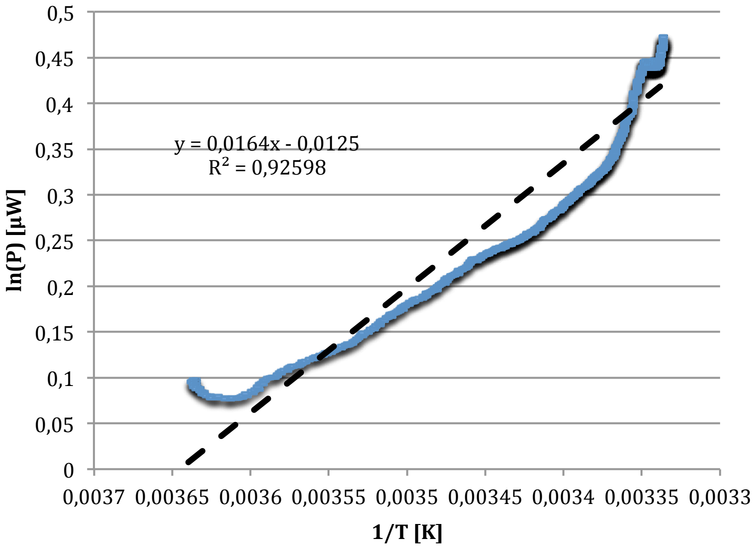

Figure 5.6 - MFC response to temperature dynamic variation over a decrement of 26°C.

Once more biofilm within MFC was promptly responding to dynamical temperature variation, this time represented is an increasing interval, from ~1,5°C to 26°C.

Still precision with fitting Arrhenius equation was high: $R^2 = 0.92$, a big result if considering that this time the interval tested was 2.5 times bigger.

The equation set used to achieve Arrhenius equation:

$$P = A\, e^{\left(\frac{-E_a}{RT}\right)}$$

if set as natural logarithms:

$$\ln P = -\frac{E_a}{R} \cdot \frac{1}{T} + \ln A$$

will match the same form of a linear equation:

$$y = mx + b$$

where:

$$A = e^b, \qquad m = -\frac{E_a}{R}, \qquad x = \frac{1}{T}$$

Also a fitting of static values for Power-Temperature has been done, reading steady state P-T values. It hasn't been utilized a very big and well organized interval of temperature, therefore the fitting appear sparse, still conserving a discrete precision.

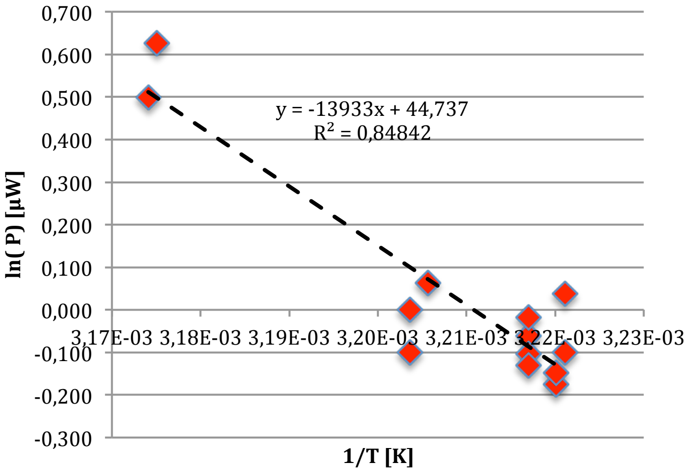

Figure 5.7 - Arrhenius plot for static temperature sensing data.

5.2.4 Conclusions

This significant experience of temperature sensing utilizing a bacterial colony was a clear sign that microbial fuel cells could be used within a new logic of living robots. More precisely MFCs built to be alive-sensors, able to give precise and fast feedbacks to stimulation from the outside world.
Among the main requirements of new concept living sensors, there is the ability of reproduce stable results for long period of time, as long as their life condition is maintained stable.
With this experiment in fact it has been demonstrated that MFCs were working within the metabolic profile of bacterial colonies, as they were able to return at one initial stable condition (e.g. the ambient temperature) once temperature solicitation terminated. Their power output was linearly and correctly corresponding to the imposed external temperature within a range of 0°C and 40°C, within indeed the metabolic interval.
This condition was not of secondary importance as with wrong temperatures (too high) the biofilm could occur into type mutations or even death.

Possible application of this kind of sensor will be the protection of living robots from too high temperature sources, potentially dangerous situation, or oppositely to spot the source of heat/cold. Those results should be considered concretely possible, as it was also demonstrated a very fast response (Figure 5.4, second peak), almost immediate, to strong temperature variations.
Once more thinking about this new conception of MFC as living sensors, it is amazing to underline their unique ability among all other sensors, to be carrying out a function whilst also contributing to the power balance of their system.

Chapter 6 Summary of the results

During the six months spent at the Bristol Robotic Laboratory with the Bioenergy & Self-sustainable group, the work on Microbial fuel cells has been developed through many different experiments that brought extremely encouraging improvements, both in the technology and in the knowledge of MFCs.

The path followed has been well structured and went through an initial period of technological improvements, where the learning of procedures such as polarizations, stabilization, feeding of a stack, COD analysis and others were practically associated with testing of new materials and configurations of MFC stack.

Cathode substitutions with MPL first and AC later brought big improvements in the performance of the cathode and the membrane, indispensable elements to complete the main reactions and increase the power output, here attesting its increase in performances around +40%.

Other actions were also carried to enhance MFC stability and develop their ability to provide a durable and constant output: with the hydration experiment, the adoption of plastic lids over the cathode volume helped preventing the undesired and penalizing phenomena of cathode-membrane dehydration, which was allowing MFC to provide maximum power just for a limited timespan of 3-4 hours maximum. Data here showed an improved in power output stability from hours to days without any needs of hydration and even though unvaried voltage. Hydration was also drastically reduced from continuous dripping to a single dose within days.

Struvite generation and collection has been faced within this dissertation, both with chemical analysis assuring the composition of this valuable precipitation of urine, and both by engineering solution to preserve the functionality and integrity the stack, avoiding, with collection devices, blockage of the flow and disruption of the main functionality.

After this first part of study, the increased performance of the whole stack allowed to set up evaluation of some key parameters such as dependence of power output from the flow rate, a two months length experiment which showed how this dimension of fuel cells needs slow flow rates (big HRTs) to get the most from the substrate. Also with this experiment was possible to underline the contribution of an increasing number of units to the power production, useful information for the future applications with big numbers of units.

Carbon consumption within urine has been deeply analysed both in the flow rate experiment and in a second experiment where COD consumption was related to different levels of solicitation of the substrate (by applying different resistances). Here results showed a linear relationship between the initial COD content within the substrate and its level of consumption once processed through the stack. Also relationship within COD consumed and loads evidenced that MFCs were able to produce a bigger amount of energy with a constant amount of organic compound if they were at their optimal load condition.

The long journey within MFCs found its final arriving point with the application of those MFCs, working together as team unit, in order to satisfy real world tasks: first the powering of a small pump, symbolic demonstration of a possible and reachable self-sustainability of the stack, where MFCs will be able to provide energy for their own survival.

Completely different task, but still very innovative and successful, was the employment of MFCs as living sensors, delivering temperature measurements real-time. Here also studies of the data showed the ability of MFCs to work repeatedly and steadily within a certain range of temperatures, even demonstrating the ability of increase their power if necessary, responding to thermal stimulations.

Results in detail

This section will cover all the improvements achieved within this work, composing a detailed report of innovations and knowledge gained.

6.1 Power output increments

Cathode substitution: from CV to MPL

- Important technological progress.
- Improved power output (not quantifiable due to different feedstock utilized with the two different materials).
- Drastic reduction of external hydration rate at the cathode side.

Improvements within CV-MPL cathode substitution derived from:

- Higher power density thanks to reduced cathode surface areas.
- Increased micro-porosity of the layer gave bigger catalytic surface to enhance catalysis rates of the reaction.
- Single layer play as advantage from packed multi-layer if water-management transport is considered.
- Increased property of hydrophobicity of the surface, allowed a strong back-pressure flow in the case of MPL utilization that keeps the membrane completely hydrated, lowering the ohmic losses, and giving clear and defined 3 phase interfaces zone where the ORR is enhanced. At the contrary for CV, the water formed or transported for electro-osmotic drag and diffusion get adsorbed by the thick multiple layers which do not create any back pressure flow.

Cathode substitution: from MPL to AC

- Single unit power increment, on average: + 36% power, with few peaks reaching +65%. Data referring over the whole group of 18 MFCs where MPL cathode has been substituted by AC.
- Stack total power increment from CV: + 237% over a 6 unit stack.
- Stack total power increment from MPL: + 53% over a 18 unit stack (see Figure 6.1).

- External hydration rate at the cathode side almost ceased (hydration rate of days).
- Consistency in almost constant improvement on each MFC, and similar R_{int}.

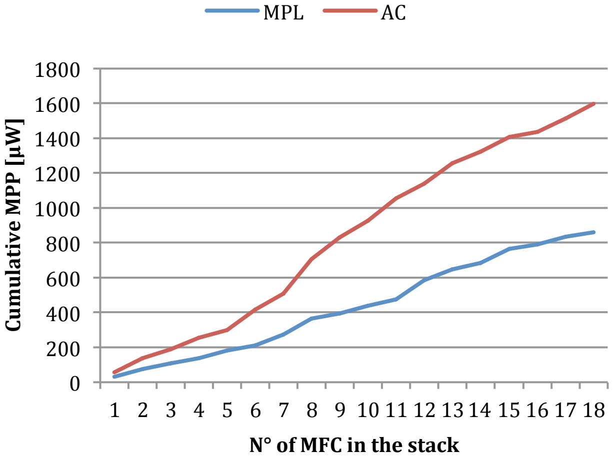

Figure 6.1 - Cumulative power output over 18 MFCs with MPL and AC cathodes.

Improvements within CV-MPL cathode substitution derived from:

- Improvement of fabrication methods for this material, highlighting as important constructive variables the pressure applied to AC particles and the treatment temperature.
- Better contact among the AC particles due to production pressing process optimization, and consequently a reduction of materials ohmic resistance.
- Better management of treatment temperature, in order to optimize hydrophobic properties of the material. The PTFE is used within this cathode as a hydrophobic and binding agent: hydrophobic properties are fundamental to the MFC functioning, as they will enhance membrane hydration.

6.2 Power stability enhancements

Adoption of covering lids and rings over cathode

- Incremented duration of high power delivery (between 75-80% of max power recorded), from initial 3-4 hour (fast decreasing) to more than 24-48 hour stable and long lasting power output.
- Improved water mass transport phenomena at the cathode side allowed formation of water droplets.
- Hydration rate further decreased till hydration not required for around 5-6 days.

Improvements within the sealing of cathode chambers by installing acrylic lids derived from:

- Annulment of antagonizing effects of room high temperature and diffusion mass transfer due to different of concentration between the humid cathode and the ambient air, by creating a micro-climate with enhanced humidity within cathode chamber.
- Increased pressure over cathode-membrane improved contact between those two, allowing higher ions transport and better hydration condition of the membrane. Water more constantly present was responsible for constant delivery of power, as the three-phase interface that need to occur between the membrane and the cathode, in order to allow the ORR to be completed, when water mass transport is missing, will miss the liquid phase responsible for the proton supply, nullifying the power production.

Struvite analysis and filtration systems

- Verified the presence of Struvite from urine as a valuable sub-product to be extracted;
- Decreased risk of hydraulic system failure. Testing of different devices apt as sedimentation chambers/column to join to MFCs stack: effective design for struvite filtration was allowing struvite not to enter the system preventing partially blockages and explosion of hydraulic connection.

Improvements within this section were brought by introducing complementary devices to the MFCs stack. Effective accumulation of struvite from aging urine was obtained by flowing the main stream through a vertical column settler, well engineered apparatus but with high HRT.

A second valid solution was obtained with floating board able to allow suction of urine right few centimetres under the surface level.

6.3 Improved knowledge with MFC stacks

Relationship between Flow rate and Power

- The highest power is obtained at the slowest flow rate: at 0.5 rpm the power from 18 MFCs has been measured as +73% bigger than 1 rpm (Figure 4.3) and so on with decreasing gaps between the other flow rates.

Slow flow rates have higher HRT, with the direct consequence of giving more time to consume the organic matter available, logically resulting in more electrons released by the biofilm and, as a consequence, more power.

- A map of how power output changes by adding more units has been outlined, for different flow rate: the addition of more unit is more effective at slow flow rates, in term of gaining more power.

At slow flow rates units are giving decreasing contribution from the first feed to the last, symbol of a reducing availability of oxidizable substrate. Yet their total power is much higher than at fast flows, indicating that high HRT plays favourably.

At fast flow rate, power production by each unit is uniform and lower, as demonstrating that triplets don't have enough time to subtract all the available carbons. It couldn't be excluded anyway that with a very big number of MFCs, fast flow rates would be favouring more MFCs to be reached by utilizable substrate, than overtaking slow-flows powers.

- A specular map of how power output changes at different flow rates, for stack of different dimensions, was also calculated: the more the stack is big, the more its power is influenced by changes with the flow rate (Figure 4.4). Almost no influence by the flow rate if the stack is very small (3 MFCs).

It was possible to underline with this analysis, an aspect of "team work" made by MFCs connected in series, in order to break down carbon compounds and make them reaching the better condition for the absorption and consume by following units. Following this logic, it should appear clear why there is no difference on power produced by only 3 MFC, at any flow rate, but instead, the more units are added, the more this difference in major power at different flow rates increase: units are taking advantages on substrate already broken down.

- The problem of accumulating debris (struvite crystals) within the anode chamber was not of secondary importance and it was possibly affecting the power production: this phenomenon, time by time was creating a thicker layer over the bacteria biofilm, lowering the optimal surface exposition to the flow, and decreasing the ability of absorbing nutrients. As this struvite sand could form agglomerates with fluid pressure, it could really become a shield to urine contact with bacteria.

Relationship between Flow rate and COD reduction

- The first of main goals reached, was to quantify how much organic compounds from urine could be reduced by a big number of MFCs. With 18 MFCs connected in series, the maximum reduction obtained was of 3500 mg C/l, equivalent to 40% of the initial content (~67% of the total oxidizable).
- No evident relationship between total COD oxidized and flow rate, as total reduction wasn't following any regular pattern. The best cumulative reduction was obtained at 1 rpm, which attain to the logic of high HRT required, but wasn't at 0.5 rpm.
- Interesting results were shown analysing the total COD reduced by varying the flow rate, for different dimensions of stacks (Figure 4.10). COD reduced was always increasing for each different stack at 1 rpm, showing a repeated and not overlapping pattern of increment before 1 rpm and decrement after. Curves this time were resulting in a regular order, showing that advantages in COD reduction were brought by increasing the number of units connected.
- Despite those conclusions, the experience rose doubts about the effectiveness of measuring units of carbon reduced to quantify "how good" was working the stack, as no match was evinced between power and reduced carbon.

Relationship between Loads and COD reduction

The experiment here involved revealed to be a key experience for the better understanding of COD measurements within microbial fuel cells and MFC stacks.

- Relation between reduced COD and Power (Load): as expressing a sort of conversion factor (previously indicated with CCF), it has been proofed that at optimal load configuration, a stack of MFC could produce a higher amount of power from a fixed amount of carbon available. At optimal load, conversion factor is proofed to be maximum (Figure 4.14).
- Relation between initial COD content (CIC) and power: it has been shown that it isn't necessary a high level of carbon content to generate the bigger amount of power.

- Talk about unit of COD reduced is not significant as same mg_C/l reduced could be obtained with very different loads.
- Higher COD reduction occurred always when CIC >11000 units, instead lower reductions are more likely verified with CIC around 7000-8000 mg C/l. These data hence showed the strong relation within initial CIC and COD reduced, resulting in an approximated linear relation.

Maximum COD reduction from a single MFC

- Measured maximum COD reduction with infinite HRT (Batch mode) was on average 1700 mgC/l, equivalent to 18% of initial content
- The theoretical 60% COD reduction reported from other studies was here never recorded.

Despite the average consumption just reported, this experience once more, as flow rate experiment over a whole stack, as cod vs. load experiment over a triplet, underline the fact that soluble COD here measured, do not express the whole carbon consumed and it is therefore not reliable to the power production itself.

- Data here showed that almost equal power output, recorded instead an almost 95% different COD reduction. Therefore, volatile COD reduction itself could not be used as only index to quantify quality of MFC performance, as instead it could be the power.
- Consistency in the behaviour: the whole energy available through a fixed volume of urine, was consumed within 48 hours, with no further variations from the minimum level of power.

6.4 Real world application of MFCs

MFCs as useful source of energy

- Successful actuation of a small pump, showing that application of MFC stacks for practical application is possible and also it is opening the road toward the development of fully-autonomous and self-sustaining MFC systems where ideally the system can provide autonomously for energy required for its vital function, for example the one required to power a pump and deliver new fresh substrate.

MFCs as living sensors: the temperature sensing

- Extremely high correspondence was shown by MFC power output in response to temperature variation, over big intervals of temperature (± 25°C), both ascending and descending. Was hence demonstrated the possibility of employing MFCs as biological-thermo sensors, inserting them into a more vast context of living sensors and robots.
- Arrhenius equation was proof to fit perfectly (R^2 around 0.97) MFC beahiuor while sensing temperature variation, also demonstrating a possibility of doubling the power output of MFCs, just regulating a more favourable environment temperature.

MFCs as waste-purification units

- Even if never directly developed within this study, clarification of fed substrate was constantly observed at the output of the stack, along this study. This also confirmed the ability of MFCs to decrease the organic compounds within the waste and purify the last.

Conclusions & Future views

Within this dissertation, a long journey inside the Microbial Fuel Cells universe has been accomplished, rich of improvements and successes and yet, the limited amount of time left so much room for further developments and topics to be explored.

Despite this technology is probably still far from mass commercialization, it really deserve to be developed and trusted as the signs of big potentiality were shown in any practical experiment within this thesis.

The knowledge and understanding developed "on-the-way" was every time opening new doors to be explored and allowing going deeper in the understanding of phenomena, refining and improving results already obtained. Every time an experiment was concluded, analysis of results was showing a possible better way to do it, or a different technique to duplicate, to confirm with more certainty the achievements.

This therefore has to be an encouragement to new students and future researchers to proceed and accept the challenge of improve Microbial Fuel Cells.

This thesis, for me has been an extraordinary chance to be first line in research even if my contribution was the smallest and most elementary one. To be at the edge of known and unknown, working day by day with both instruments and ideas, planning and solving new experiments and issues was really paying back all the time dedicated.

It has been indeed such a good feeling and honourable duty to put my brain, time and dedication in service of a science useful to people, able to go further than the mere laboratory bench and, let me think about the infinite utility in a not too far future of this bio-technology. This is really something capable of producing clean electricity and clear the wastes at the same time, to be simple and cheap, to respond to external stimulation and cooperate with similar units.

It has been a skilful experience for me, really enriching from the practical point of view, since from day zero I was working hands-on the project, learning a lot about the technology of MFCs as well as learning from tutors and colleagues how to plan experiments, where "to look" for results; a lot of personal and professional enrichment comparing myself with the most dedicate, smart and passionate people amongst the whole Britannic researchers, at the BRL.

Thank you Yannis, John, George, Iwona, Sun, Alexis, Jonathan, Lily and all the other colleagues!

Future developments

As I hope that my work will be really a “brick” to build and move forward the knowledge of MFC, I would like to suggest possible ways to proceed on what has been done so far.

It is opinion of the researcher that for a correct understanding and real time check-up of MFC behaviour, consumptions occurring are key-data. As this study shown a big unreliability of COD, than individuation and testing of different methods of measuring the carbon consumptions would be really useful. In order to verify this, and to allow the usage of real urine (with all its variability), a same batch could be used to feed in parallel and simultaneously a good number of MFCs. This way data will be available, avoiding variability of urine composition.

Concerning the theme of best flow rate, results were affected by debris sedimentation within the anodes, therefore, any repetition done in future, e.g. for other designs, should consider the care of clearing anode chambers after each stage, for example rinsing the MFC with fresh urine.

If the interest will be about design improvement instead, weak point was highlighted for example within the electric connectors, often giving false readings. Contact point between the cathode veil and the metallic wire, could damage relatively fast and it is hard for the researcher to see it from the outside, especially with new design of closed chambers. As already done by other researchers, thinking about melting a metallic very thin mesh structure within the cathode layers could be evaluated.

Another weak point has to be individuated in the imperfect contact between cathode and membrane: even few decimals of millimetres could be a huge distance for ions and electrons. It has to be tested any mechanical pressuring, or even better, any thermal process able to join together those two elements, making their contact perfect.

Experiment involving effects of urine aging, such as record the aging of stored urine samples, or such as power production with different aging of urine could have been some important data for real installation of MFCs.

Also, one other way to understand parameters connected with power production optimization could be to measure the different time required to charge capacitors, at different flow rates. Moving the flow rate experiment from a theoretical to a practice level.

Practical advises for future students

I feel my project as just a small chunk of what could be done with MFCs, and especially concerning what I've done so far, I would suggest future students to proceed in the direction of investigating power output increments, testing new designs or pressing techniques to ensure perfect adhesion between membrane and cathode, making contact surfaces more resistant but yet favourable to their main function. I also suggest to carefully ensuring every time a new experiment is planned, to have perfect comparable power outputs from each units as otherwise a weak unit will falsify the results.

Every time one idea comes and an experiment has to be planned, always think about, what I will show in my results? Which data do I need to make my experiment correct, and more than else, utilizable? How many variables am I taking into account, if more than one, will I be able to exclude them from each other?

To future student it has to be said that it is not always easy way to the end of experiments: there will be days with things not working or bad results, but despite this, methodical annotation of all the actions and data available, and constant monitoring and cross-processing of these data, will help in the process, giving real time updates of your work and chances of corrections on the go, giving finally consistent results. Don't wait till the end of the experiment to see what your data are showing!

And… Last but best suggestion, never, never change conditions when an experiment is already started.

Hope to you a great experience with Microbial Fuel Cell and the great team at BRL, as it has been for me!

Annex

A.1. Polarization

What is measured

A polarization test was conducted in order to find the Maximum Power Transfer (MPT) of a Fuel Cell, as the theoretical best power output, associated with the application of the optimal resistance.

The polarization was performed by connecting a variable resistor to each triplet, starting from the open circuit voltage, by decreasing the resistance at fixed time intervals, and following a precise series.

The voltage output was recorded. It was so far possible to calculate the power output at each resistance value, and discover which load-set allow the MFC to work at its best condition, delivering the maximum amount of power. This condition was identified as MPT.

It was of very interest, to be able to know this particular functioning condition, because it was always wanted to run any test or operation at the MFC best performances.

For example it could be useful to see if after a change of materials, flow rate or type of feeding, any increase or decrease of the power output occurred with MFC.

The procedure

A variable resistor was connected to each triplet. It was necessary to start a polarization from the highest value of voltage that an MFC can reach, which corresponded to the *open circuit voltage* (OCV). In order to do so, it was preventively requested to unload the triplet for at least 12-16 h, allowing the MFC to reach the voltage plateau.

The equivalent condition for OCV, by utilizing a variable resistor, was the infinite resistance; for this reason, the highest value of resistance available on the instrument was set as initial value of the polarization.

A series of decreasing resistances was applied, switching the resistance every 3 minutes; this time was considered long enough to allow voltage to stabilize.

Chart 6.1 - Resistance sequence for polarization.

Polarization resistance sequence			
999999 Ω	12000 Ω	900 Ω	90 Ω
30000 Ω	10000 Ω	800 Ω	80 Ω
28000 Ω	8000 Ω	700 Ω	70 Ω
26000 Ω	7000 Ω	600 Ω	60 Ω
24000 Ω	6000 Ω	500 Ω	50 Ω
22000 Ω	5000 Ω	400 Ω	40 Ω
20000 Ω	4000 Ω	300 Ω	30 Ω
18000 Ω	3000 Ω	200 Ω	20 Ω
16000 Ω	2000 Ω	100 Ω	10 Ω
14000 Ω	1000 Ω		

A *PicoLog Data-Logging* channel was also connected to each triplet, recording one value every 30 seconds. This timing allowed recording 6 value of voltage; the last 4 stable readings were considered as useful data and an average voltage was calculated.

By combining the voltage and the resistance applied, power and current were calculated for each resistance value of the list.

$$I = \frac{V}{R}$$

$$P = V \cdot I = \frac{V^2}{R}$$

The polarization procedure could be conducted both automatically, by the usage of *Mr.Beam polarization device*, both manually. During all this study only manual polarizations were performed, so it was very important to respect the synchronization between recording intervals and switch of resistance in order to keep the measurement accurate.

A.2. COD Measurement

What is measured?

COD stands for Chemical Oxygen Demand, or amount of oxygen required to completely oxidizing, through chemical process, all the organic compounds contained within a liquid sample.

COD is expressed as *mg*O_2*/l*. For this reason it become an indirect method to measure the amount of Carbon (C) contained in a sample.

Chemical Oxygen Demand is often used as indication of the total organic content of water or other biological liquids. In detail, it is a measure of the amount of dichromate that is reduced by oxidation of the organics. The oxidation of most organic compounds by dichromate is 95 to 100% of the theoretical value; however, in the case of urine, ammonia and urea are among the compounds that are not oxidized by dichromate.

Since urine contains a large amount of urea, ammonia and amines, its COD values would be expected to run considerably below the total organic content of urine, as proofed in previous studies [14].

By measuring the initial and final value of COD, of a urine sample going through a MFC, it is possible to estimate the amount of COD consumed by the fuel cell biofilm, the ability of the MFC to reduce organic compounds within the given feedstock.

The aim of the COD analysis conducted within this study is to measure the ability of each triplet, and of the overall stack, to reduce the substrate carbon content

The procedure: Slow Sampling

The sample of urine was collected while the main stack was completely empty and the flow restarted and going to fill it up again.

Before start the sampling it was necessary to stop the normal flow and, by opening the tap at the bottom (Tap 0), the substrate was allowed to flow out the stack by gravity.

Additionally, a 50 ml syringe, filled with air, was blown from the outlet of the stack, with the intent of clearing as much as possible the internal volume of the stack (anodic chambers and tubing) from residual anolyte. The operation was repeated twice.

As the stack was completely empty, the fresh substrate was pumped at position Tap 0 and the first sample of urine, corresponding to fresh substrate was collect upstream the stack. Samples will than be taken from the first to the last tap, by the time the flow will reach each tap, filling progressively each triplet. A different volume of fluid substrate was required depending on the magnitude of the reduction that has to be measured.

This procedure of analysing COD consumption over a stack of fuel cells was considered the ideal one because collecting the sample by the time it flows up, along the series of MFCs, allowed to obtain the sequential reduction, on the same, initial, volume of substrate. It was so measured the ability of each triplet to reduce a ideally fixed volume of urine, with decreasing carbon content.

With the experience it was proved that this method could cause wrong readings because debris into anolyte chamber could mix with fresh urine and affect heavily the COD value, compromising the measurement.

The procedure: Fast Sampling

The sample of urine was collected while the main stack was on normal operative conditions (system filled with urine, flowing regularly) and stable power output, starting from the end of the stack to the beginning.

Starting from the far end of the stack, and acting on the last T-connector, the flow outgoing triplet n°6 was deviated and collected into a vial. Than sampling port was closed without having so far influenced the rest of the MFCs upstream. Sampling was repeated for tap 5, 4, 3, and so on till the inlet (Tap 0).

It was appropriate to start sampling from the end, because in this way all the following MFCs were keeping their stability in term of turbulence and internal debris agitation.

The fast-sample collection procedure allowed a real time and realistic monitoring of COD levels along the stack.

Processing the samples

The COD reduction was measured in mgO_2/l, equivalents to the *mgC/l* reduced; depending on the order of the C reduction expected, it is possible to use different levels of the chemical reagent.

These different levels could be used to increase the definition of a reading: even if the level of carbon was high, by adopting a dilution at different levels, it was possible to enlarge the scale of the variation.

High Range levels

The reagents utilized can process organic matter containing from 15000 *mgC/l* to 0 *mgC/l*; this range is correct when big reductions of COD have to be measured, and it isn’t required a fine scale.

With high range COD vials, the sample was prepared starting from approx. 2 ml of urine (each coming from a different tap, as explained before) collected into sterile vials and labelled.

Each sample was filtered with sterile, single usage, filter to allow only the soluble carbon contained in solution, to be measured, and the resulting collected into a glass vial.

An amount of 2 µl of filtered urine was added into the HR reagent, contained into single-usage glass vials, and the whole was stirred gently.

Also another same vial was mixed with 2 µl of deionized water, in order to create a calibration vial.

The sample was now heated till the temperature of 150° C for 120 min. After this process the vial had assumed a colouring between deep yellow and deep green, and was now left cooling for approx. 30 min.

Once the sample was cooled, it was ready to be measured by inserting it into a colorimeter appositely calibrated to correlate the colour scale with the amount of C contained.

In order to work properly, the colorimeter had to be calibrated with an equivalent HR COD vial, containing just deionized water.

Medium Range levels

The reagents utilized in this case can measure samples containing between 1500 *mgC/l* and 0 *mgC/l.* It is suitable to use this range of vials when the difference between two measurements is small and it is wanted to increase the definition of that interval.

It was here required the availability of a bigger amount of sample, due to the necessity of adding to the COD MR vial, an amount of diluted solution equal to 2 *ml.*

The sample was diluted by 1/10, and 0.5 *ml* was added to 4.5 *ml* of deionized water, and all stirred well.

The amount of 2 *ml* of sample diluted was so added to medium range reagents, and the vials was than processed for 120 min. at 150°C. Same procedure to read the final value of COD inside the sample, this time utilizing a MR control vials with 2 *ml* of deionized water.

A.3. Pump calibration

Urine density

The density of the urine was required to calculate the flow rate.

To calculate an average value for urine density, a vial with volumetric graduation was used to collect 5 mL of urine, which correspond to a volume of 5 cm^3. The sample and the vial were weighted on a precision scale and the tare of the vial (known by weighting it when empty) was subtracted to the total. By doing so, it was measured the mass of 5mL of urine.

It was calculated the density of the urine:

$$\rho = \frac{m}{V}$$

To obtain a more precise value, the sampling was repeated four times and the average calculated.

Urine density [kg/m^3 at 20° C]	978

Pump flow rate

Knowing the flow rate delivered by the pump utilized, was required within the flow rate experiment, in order to be able to calculate the *hydraulic retention time* (*HRT*) of MFCs, triplets and overall stack.

Watson & Marlow mod.205 S pump was utilized during all the experiment as main propeller for the feedstock. This device expresses its rotational speed as *RPM*, it was so necessary to apply a conversion if the flow rate was desired.

Flow rate of pump *Watson & Marlow mod.205 S* was calculated by measuring the amount of fluid delivered over a known time length; at constant *RPM*, it could vary depending on fluid density. In this case, urine density was known and assumed constant.

The urine was collected into vials of known weight, for an established amount of time. After that time passed, the pump was stopped and the sample weighted with a precision scale, so the mass of the liquid was now known. Calculating the volume through density and mass, it was possible to express it as *ml*; dividing by the elapsed time, it gave the flow rate, expressed in *ml/s,* at that *RPM.*

The procedure was repeated for water and urine, resulting in a slight difference at high flow rate, due to a small difference in fluid density.

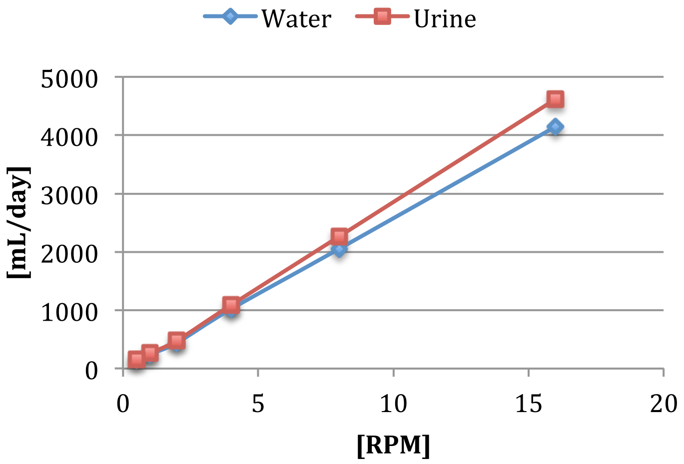

Figure 6.2 - Watson&Marlow peristaltic pump flow rate, for urine and water.

Chart 6.2 - Water: flow rates-rpm conversion chart for Watson&Marlow peristaltic pump (ρ = 998.2071 kg/m3 at 20 °C).

RPM	[mL / s]	[mL / hour]	[mL / day]
0.5	0.00167	6.012	144.3
1	0.00284	10.224	245.38
2	0.00514	18.504	444.1
4	0.01183	42.59	1022.2
8	0.02378	85.61	2054.6
16	0.04800	172.8	4147.2

Chart 6.3 - Urine: flow rates-rpm conversion chart for Watson &Marlow peristaltic pump (ρ = 978.00 kg/m3 at 20 °C)

RPM	[mL / s]	[mL / hour]	[mL / day]
0.5	0.00178	6.41	153.8
1	0.00307	11.052	265.3
2	0.00558	20.09	482.2
4	0.01261	45.4	1089.5
8	0.02632	94.75	2274.1
16	0.05351	192.64	4623.3

A.4. Measurement of stack volumes

Once the flow rate delivered was known, it was possible to proceed with the calculation of all stack internal volumes.

The procedure adopted to do so, was very empirical but it was enough to have a quite good idea of *HRTs*, and was finally resulting with truthful values.

To measure the main stack volumes (composed by 18 fuel cells, short pipes between each MFC and long pipe with T-connector between each subsequent triplet) it was measured the time required to the volumes to fill at an established flow rate. The measurement of times was done with manual chronometer. It was done with few of the flow rates required by "flow rate experiment", in order to make an average of the results and take in account possible errors or variations due to dilatation of membranes or piping by pressure. The stack was previously emptied by blowing air through it with a 50 ml syringe.

Volumes where than determinate:

$$V = \frac{\dot{V}\ [m^3/s]}{t}$$

Charts and graphs about measured MFC volumes were previously reported in Figure 1.8 and Chart 1.3.

A.5. HRT – Hydraulic Retention Time

Once known the volumes and the flow rates delivered, it was possible to calculate the *hydraulic retention times* (*HRT*) of the stack, at each different flow rate.

This data was very important in order to estimate sampling time within COD analysis of a big stack, at very slow flow rate, when the time required to fill the stack could also reach 10-11 h.

Chart 6.4 - Main stack HRT

HRT – Hydraulic Retention Time								
	0.5 rpm	1 rpm	2 rpm	4 rpm	8 rpm	16 rpm		
$\dot{V}$ [cm^3/s]	0.001779	0.003067	0.005581	0.012611	0.026233	0.053511		
HRT [min]	606.0	351.4	193.1	85.5	41.1	20.1	All main stack	
HRT [h]	10.1	5.9	3.2	1.4	0.7	0.3		
							Triplet	MFC
HRT [min]	93.6	54.3	29.8	13.2	6.3	3.1	1	16, 17, 18
	102.0	59.2	32.5	14.4	6.9	3.4	2	19, 20, 21
	78.3	45.4	24.9	11.0	5.3	2.6	3	1, 2, 3
	79.5	46.1	25.3	11.2	5.4	2.6	4	4, 5, 6
	79.4	46.1	25.3	11.2	5.4	2.6	5	7, 8, 9
	80.8	46.9	25.8	11.4	5.5	2.7	6	10, 11, 12

List of Acronyms

Here following are reported acronyms used within this dissertation:

- **AC**: Activated Carbon (cathode)
- **CCF**: Carbon Conversion Factor
- **CEM**: Cation Exchange Membrane
- **COD**: Chemical Oxygen Demand
- **CV**: Carbon Veil (cathode)
- **MFC**: Microbial Fuel Cells
- **MPL**: Micro Porous Layer (cathode)
- **HRT**: Hydraulic Retention Time
- **OCV**: Open Circuit Voltage
- **ORR**: Oxygen Reduction Reaction

Figure credits

For figures taken from external sources and utilized within this dissertation:

1.2 – source Web Google:
http://www.mathworks.com/help/releases/R2013b/physmod/
sps/powersys/ref/fcpolarisation_curve.gif
1.4 – Solid edge views of MFC design, courtesy of Jiseon You, BRL.

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