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Combined Hydrogen and Bioethanol Production within a Microbial Electrolysis Cell (MEC)

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Abstract

The integration of bioelectrical systems to a bioethanol production plant was investigated on small scale. A reactor was constructed with the aim to be operated as a microbial fuel cell (MFC) and a microbial electrolysis cell (MEC). The reactor was characterized in terms of power output and internal resistance with *S. cerevisiae* as biocatalyst. An internal resistance of 954 Ω was measured for a maximum power output of 176 mW/m^3 anode.

Electrical currents were measured with the MFC using *S. cerevisiae* as biocatalyst. Five glucose concentrations ranging from 20 g/l to 170 g/l were tested in presence and absence of the mediator Methylene blue. The mediator enhanced the current production but for small glucose concentrations but decreased also the ethanol production yields. In a general way higher glucose concentrations increased the number of coulombs produced, this was observed in presence and absence of the mediator.

Four set-ups were tested to produce hydrogen in MEC mode. The MEC was first operated in single chamber mode, both anode and cathode were immersed in a *S. cerevisiae* fermentation, hydrogen production was not detectable but a high amount of carbon dioxide. For the second set-up a proton exchange membrane was added between the half cells and a phosphate buffer pH 7.0 was used as catholyte. 2.0 V, 3.0 V and 4.0 V were applied, traces of hydrogen were detected under 3.0 V and 4.0 V. However applying such high voltages impacted negatively ethanol production. For the third set-up HCl 0.5 M and 2.0 M were used as catholyte. 0.48ml of hydrogen was produced with HCl 0.5 M and 0.67 ml with HCl 2.0M under 1.3 V. However, we observed a drastic decrease of ethanol yields and *S. cerevisiae* viability when using such acidic catholytes. Finally, fermentation wastes were recovered at the end of a fermentation and digested in a MEC using *Shewanella oneidensis* MR1 as biocatalyst.

For the last series of experiments, the valorization of fermentation wastes through methane production was tested. Wastes were recovered from ethanolic fermentations realized with *S. cerevisiae*. The wastes were then digested in an electromethanogenesis cell having active biofilms on the electrodes. The wastes were successfully converted into methane, highest conversion rate of the COD into methane was 91.1%.

Content

1. Aim

The aim of the project was to show how bioelectrical systems can improve the productivity of a bioethanol production plant. The research team investigated the parameters that allow to combine ethanol, electricity, hydrogen and methane production.

The whole project can be divided in four main steps. The first one consisted in the construction of a bioreactor capable to be operated as a microbial fuel cell and microbial electrolysis cell. Once the reactor was built a characterization of the system was performed. A series of experiments leaded to a performance analysis of the reactor when operated as a microbial fuel cell. In the third step bioethanol was produced by glucose fermentation with the yeast *Saccharomyces cerevisiae*, while an electrical current was recovered from the cultivation. This was achieved by operating the reactor has a microbial fuel cell. By adapting the set-up of the reactor, hydrogen and bioethanol can be produced in parallel. For each configuration the impact of electrical current and hydrogen generation on the ethanol yield was studied. In the longer run the goal was to find the appropriate equilibrium between this three energy vectors in order to recover the highest performance of the system.

Another approach to integrate bioelectrical systems into bioethanol production plant is to use the wastes generated during the fermentation process as substrate to produce energy. Valorisation was achieved by adding fermentation wastes into a MEC in which *Shwanella oneidensis* MR1 was used as biocatalyst. Wastes were also digested in an electromethanogenesis cell by active biofilms formed from activated sewage sludge. In this lastest set up, fermentation wastes were converted into methane rich biogas.

2. Principle

2.1 *Saccharomyces cerevisiae* in MFCs and MECs

The yeast *Saccharomyces cerevisiae* is widely used in bioethanol production. In fact, it is well known for his ability to produce high ethanol yield from simple sugars like glucose[1] (see figure 1). *S. cerevisiae* is considered as a crabtree positive yeast since it is able to degrade carbohydrates to ethanol even in the presence of oxygen. However the fermentation pathway is privileged under anaerobic conditions or

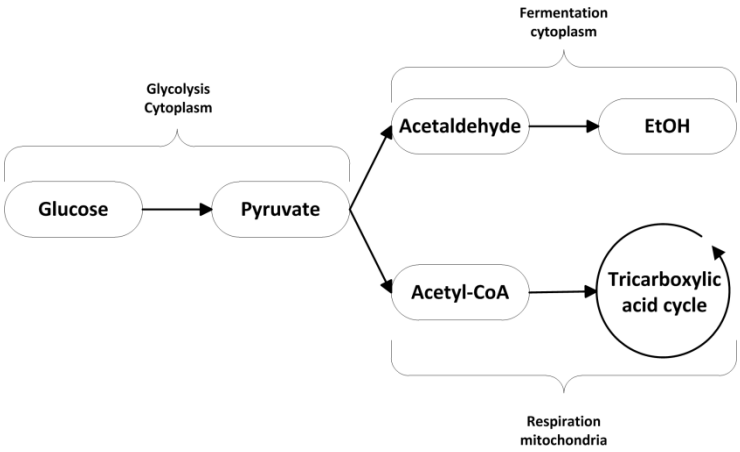

Figure 1: Glucose metabolism of *S.cerevisiae*.

when high sugar concentration creates a pyruvate overflow and a saturation of the respiration metabolism (if O_2 is present)[2].

The final electron acceptor is usually oxygen in the yeast metabolism, but in a system such as the microbial fuel cell it can be replaced by a mediator or an electrode. The Methylene Blue is a well-known mediator, it is reduced by the metabolism of the biocatalyst before being oxidized on the anode surface. The mediator is acting like an electron shuttle between the microorganism and the anode[3] when present in the medium. When no mediator is present in the medium the electrons are transferred directly from the microorganism to the anode. The electrons released at the anode travel through an external circuit to the cathode were they reduce the catholyte, the final electron acceptor. Protons are produced stoichiometrically, depending on the amount of electrons and nature of substrate. These protons flow through a proton exchange membrane to the cathode compartment in order to maintain the electro-neutrality of the system.

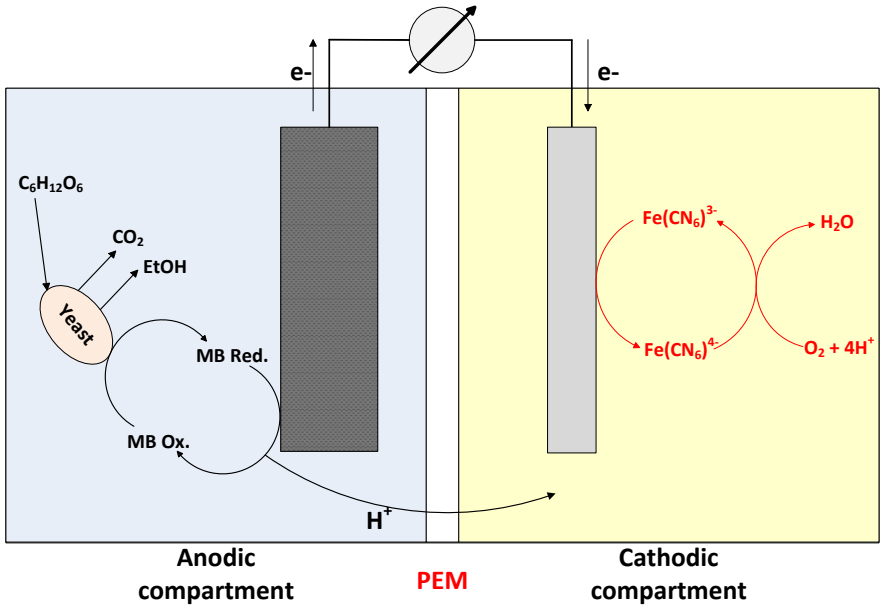

Figure 2: Microbial fuel cell principle. MB: Methylene Blue, mediator in the anode compartment. PEM: proton exchange membrane. The final electron acceptor in the cathodic compartment was potassium ferricyanide.

By removing the final electron acceptors in the cathode compartment (including oxygen) and applying a small voltage, hydrogen can be produced. This is an emerging technology known as microbial electrolysis cell. In a microbial electrolysis cell the electrons flow through an external circuit and meet the protons at the cathode. The protons are then reduced resulting in hydrogen production. The addition of an external applied voltage is required since the conversion of organic compound into hydrogen is thermodynamically unfavourable.

Anode reaction:

$$C_6H_{12}O_6 \rightarrow 6CO_2 + 24H^+ + 24e^- \qquad E_{anode} = -0.43V \qquad (1)$$

Cathode reaction:

$$2H^+ + 2e^- \rightarrow H_2 \qquad E_{cathode} = -0.414V \qquad (2)$$

The difference between the anode and cathode potential of -0.844 V indicates that the electrons won't flow spontaneously from the anodic to the cathodic compartment[4]. In order to overcome this potential difference a small electromotive force is added to the system with the help of a power supply unit.

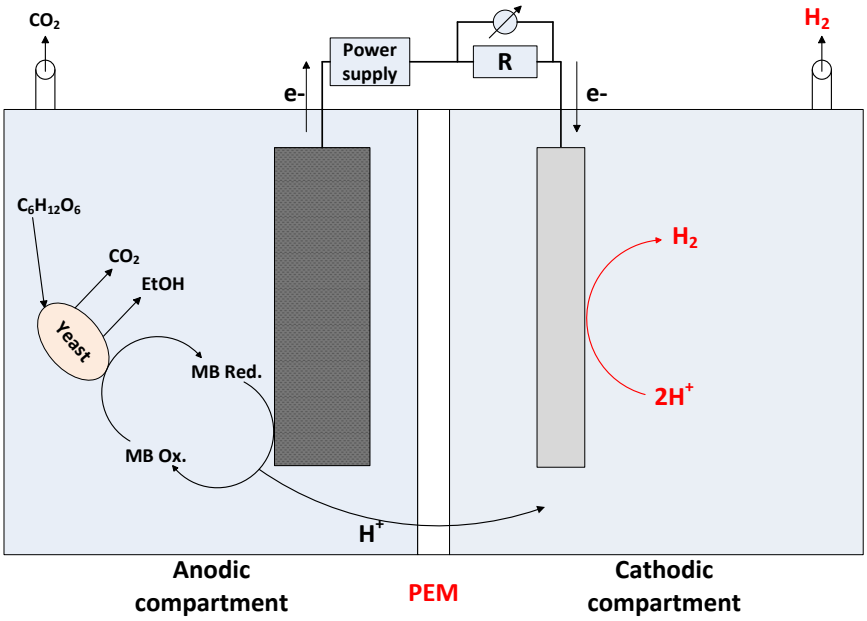

Figure 3: Microbial electrolysis cell principle. MB: Methylene Blue, mediator in the anode compartment. PEM: proton exchange membrane. R: external resistance. The final electron acceptors in the cathodic compartment are protons released in the anodic compartment.

As mentioned before a microbial fuel cell is able to produce an electrical current and this can be converted into a microbial electrolysis cell, which produces hydrogen instead of electricity. If the biocatalyst converts organic substrates such as glucose to ethanol the system produce simultaneously biohydrogen and bioethanol or electricity and bioethanol.

2.2 Electromethanogenesis

Converting the wastes produced during an ethanolic fermentation into biogas though electromethanogenesis could be an alternative to valorise bioethanol plant wastes and improve the systems performance. Both anodes and cathodes were immersed in the bulk in an electromethanogenesis cell, biofilms were formed from activated sewage sludge. As in a microbial electrolysis cells the organic matter is oxidized by a biofilm on the anode and the resulting electrons are driven through an external circuit to the cathode with the help of a small applied voltage. The biogas produced was a mix of methane and carbon dioxide. The aim of the electrodes and the applied potential is to enhance the methane production to the detriment of carbon dioxide. In fact, the applied voltage will lead to hydrogen production on the cathode according to microbial electrolysis cell principle. The reactions consist in a conversion of carbon dioxide into methane[17] through in-situ hydrogen production.

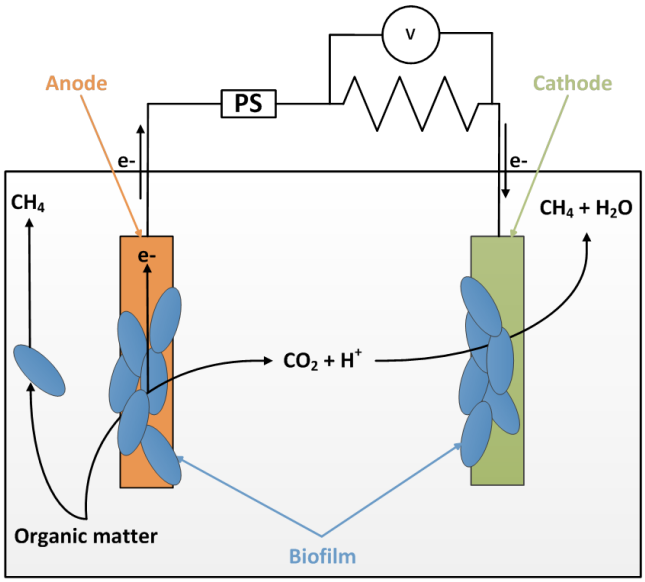

Figure 4: Methane production from fermentation wastes. Carbon dioxide is converted into methane through in-situ hydrogen production.

3. Material and methods

3.1 Bioelectric fuel cell construction

The reactor was constructed by the mechanical shop of the HES-SO Valais Sion. The bioelectric fuel cell was constructed from the beginning as a microbial electrolysis cell (MEC) with the option to be used as a microbial fuel cell (MFC). It consisted of two compartments of 500 ml each; separated by a 91 cm^2 Nafion® N117 (Ion power, Germany) proton exchange membrane (PEM). The cathodic compartment contained a nickel cathode 130 x 4 x 70 mm (length x width x height). The anodic compartment contained an anode made of reticulated vitreous carbon (RVC) with a true surface area of 1.71 m^2 (ca. 65 cm^2 cm^{-3}) for a volume of 262 cm^3. The distance between the anode and the cathode was 36 mm.

The anodic and cathodic compartments were equipped with quick connections on top and bottom. For the anodic compartment these connections were used to mix the cultivation in order to guarantee a good mass transfer between the substrate and the microorganisms and to avoid sedimentation of the microbes. The liquid was continuously taken from the bottom of the compartment and brought to the top by a peristaltic pump (Masterflex, Switzerland). For the cathodic compartment the connection in the bottom was used to remove the oxygen present in the compartment by sparging nitrogen gas 4.5 (Pangas, Switzerland) at a flow rate of 50 ml/min for 30 minutes. The connection on the top was used to collect produced gas. Gas tight tubing was connected on the top of the cathodic compartment to a milligas counter MGC-1 V3.2 PMMA (Ritter, Germany).

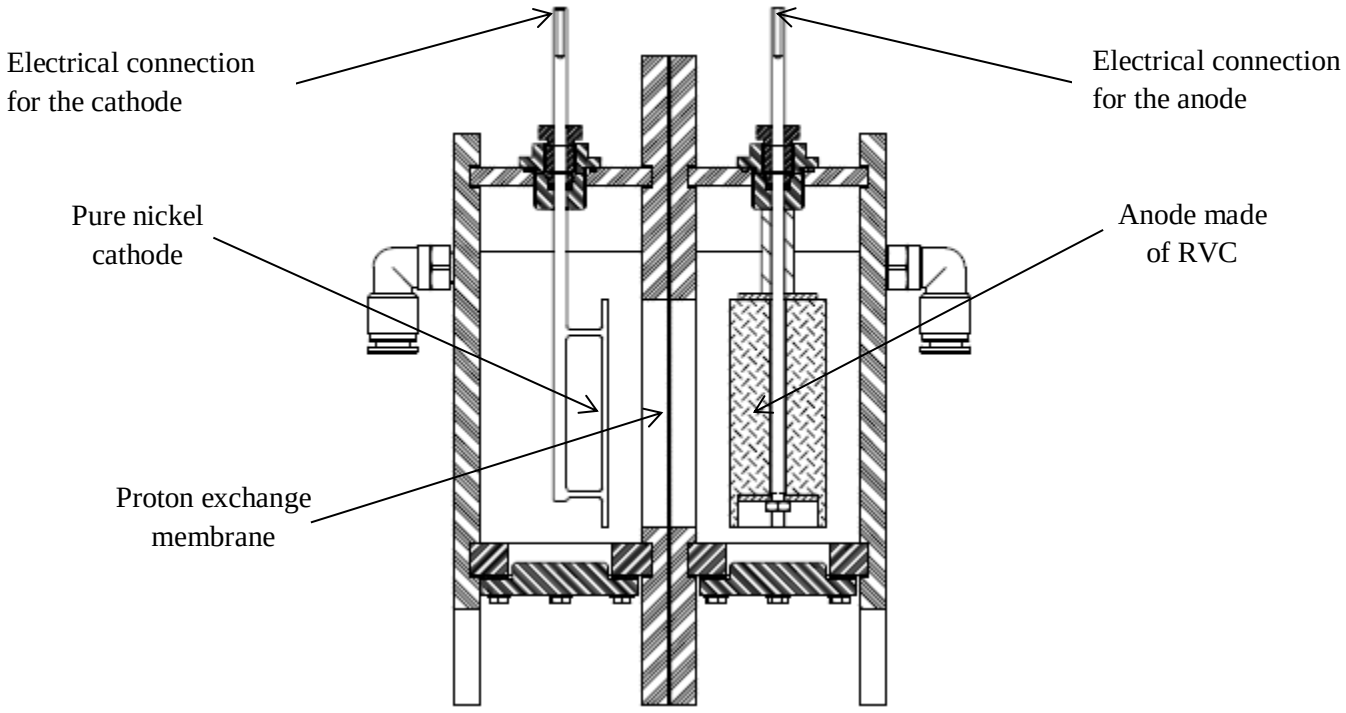

Figure 5: Set up of the fuel cell with anodic and cathodic compartments

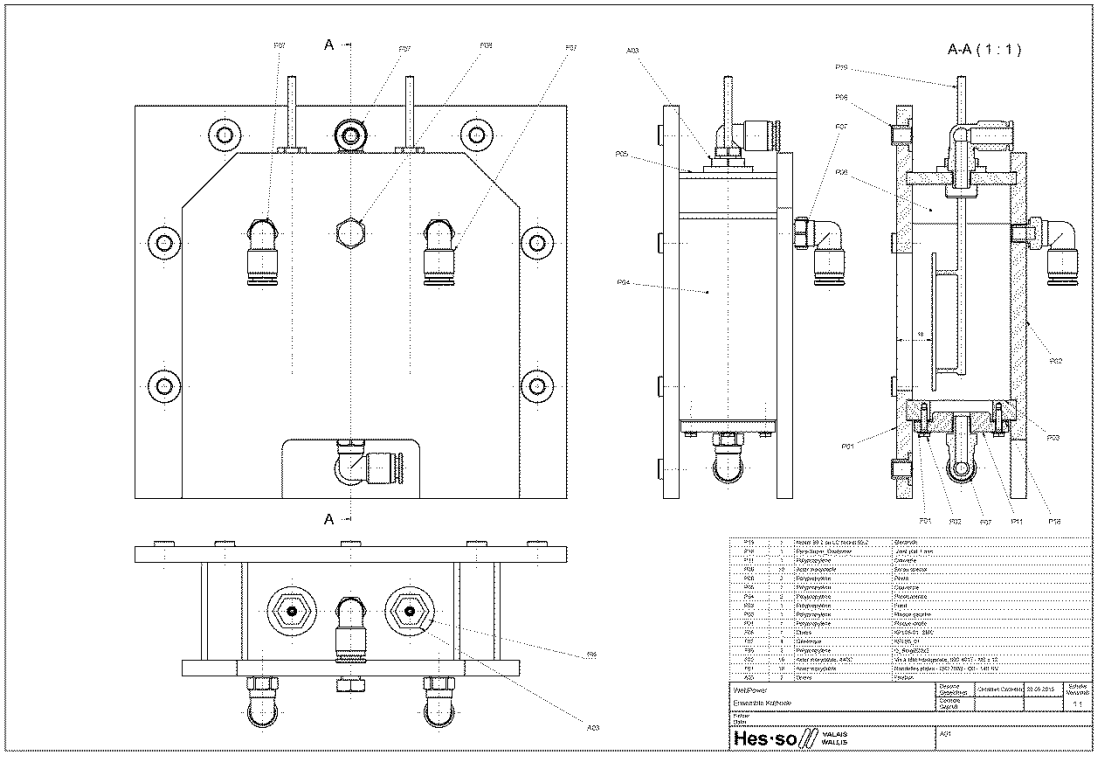

Figure 6: Plan of the cathodic compartment. The cathode including the external connections were made from pure nickel. The gas generated was collected through the quick connection on the top of the respective compartment. The quick connection on the bottom of the compartment was used to remove trace oxygen by sparging with nitrogen.

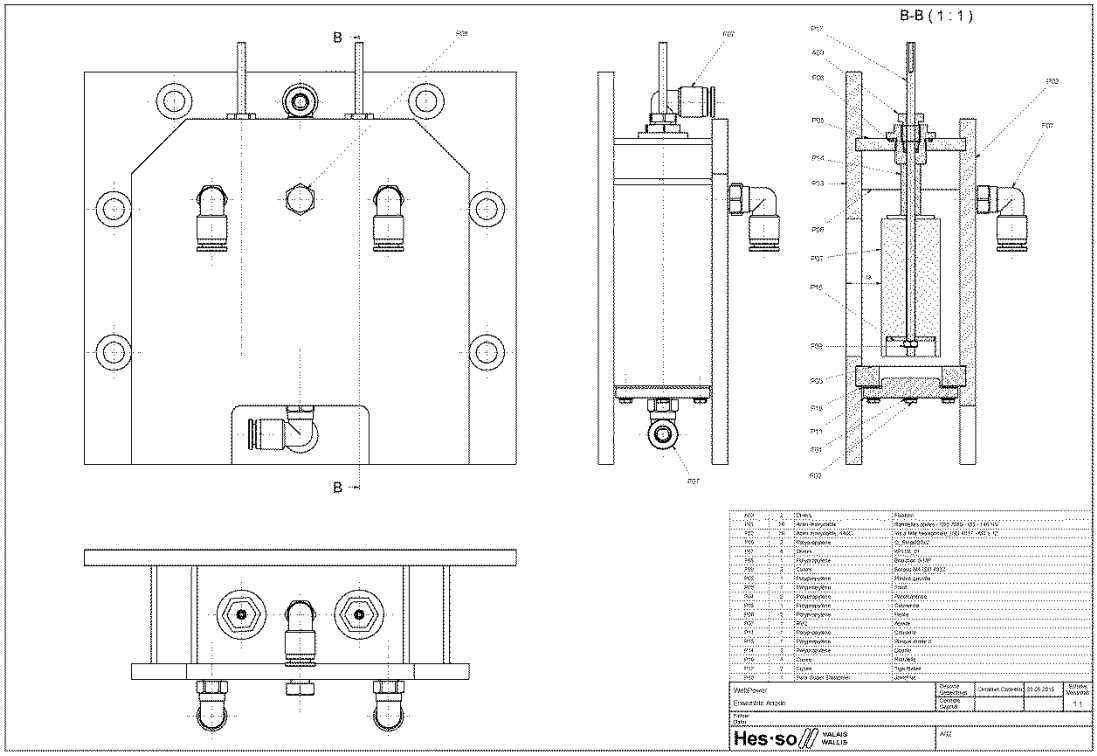

Figure 7: Plan of the anodic compartment. The anode was made from RVC, the external connections were of PTFE. The quick connection at the bottom of the compartment was connected on the top of the compartment, a peristaltic pump was added on the tube in order to mix the cultures in the anodic compartment.

3.2 Preparation of platinized cathodes

When the bioelectric fuel cell was operated as a microbial electrolysis cell the nickel cathode was covered by platinated reticulated vitreous carbon (RVC) electrodes. Platinum is a catalyst used in microbial electrolysis cell technology, his role is to enhance hydrogen production[5]. Pieces of reticulated vitreous carbon of 5.5 x 0.5 x 4 cm (length x width x height) were sputtered with pure platinum using a sputtering device Sputter Coater SCD-050 (Bal-Tec, Switzerland). The RVC electrode was inserted in the vacuum chamber and the pressure set to $8*10^{-2}$ mbar and 60 mA for 3 minutes. Before and during the sputtering Argon was injected to the vacuum chamber as protection gas.

Photo 1: The Sputter coater device used for platinum deposition on reticulated vitreous carbon electrodes.

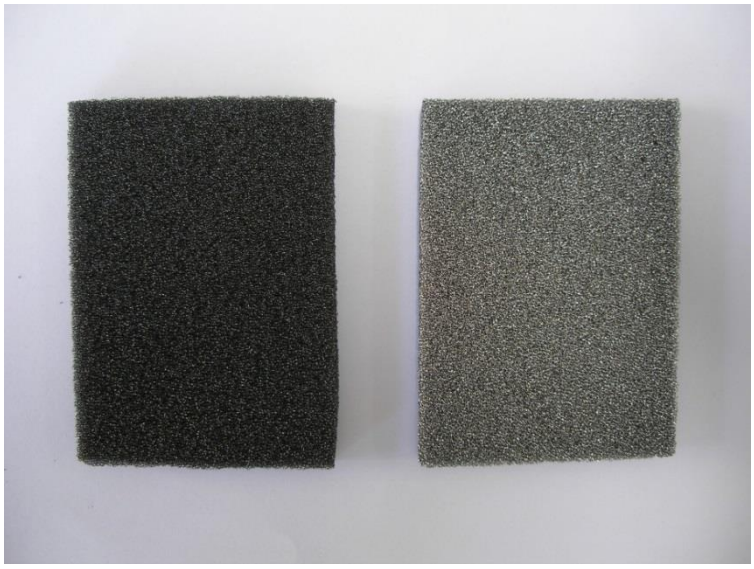

Photo 2: Reticulated vitreous carbon before (on the left) and after sputtering (on the right).

3.3 *Saccharomices cerevisiae* cultivation and ethanol yield calculation

All experiments were realised with *Saccharomyces cerevisiae* DSM 4266. The strain was stored on agar plate at 8°C. The agar plates had the following composition: 1% yeast extract (Lonza, Switzerland), 1% enzymatic hydrolysate of soybean meal (BD, USA), 2% of glucose (Cargill, Switzerland) and 15% of Agar (Fluka analytical, Switzerland). The yeasts were stored for no more than three weeks, at the end of this period a transfer to fresh agar plates was realised. The agar plates were incubated for 48 hours at 30°C before being stored.

Before the inoculation of the reactor, *S.cerevisiae* was precultivated in a shake flask containing 100 ml of the following media: 1% yeast extract, 1% enzymatic hydrolysate of soybean meal and 2% of glucose.

The precultures were inoculated with a colony from a stored agar plate and cultivated overnight at 30°C and agitated at 180 RPM.

The media used in the anodic compartment had the following composition: 1% yeast extract, 1% enzymatic hydrolysate of soybean meal, 20, 50, 70, 120 or 170 g/l of glucose. The pH was adjusted to 5 by the addition of phosphoric acid (Acros organics, Switzerland). A mediator was used in some experiments, it was 0.3 mM of Methylene Blue (Fluka, Switzerland) in used media. Before being inserted in the anodic compartment of the bioelectric fuel cell, the media was inoculated with 2% v/v *S.cerevisiae* preculture.

All media mentioned in this section were sterilized at 121°C for 20 minutes before use. The glucose and other constituents were autoclaved separately in order to avoid Maillard reactions. The glucose was added to other constituents at the end of the sterilization under a laminar air flow cabinet.

Saccharomyces cerevisiae possess the ability to convert C_6 compounds into C_2 compounds, especially ethanol through their fermentative metabolism. If we assume a complete conversion of glucose into ethanol[2, 14] 0.51 gram of ethanol is produced per gram of glucose.

$$C_6H_{12}O_6 \xrightarrow{yeast} 2\ C_2H_5OH + 2\ CO_2 \quad (3)$$

The ethanol production yields were calculated by considering 0.51g ethanol/g glucose as 100% conversion.

3.4 *Saccharomyces cerevisiae* fermentation in shake flasks for blank experiments

Blank experiments were realised in 500 ml shake flasks. 200 ml of culture medium was inoculated by *S. cerevisiae* and incubated at 25°C and 180 RPM, the flasks were closed with cellulose steristoppers. The medium used for the blank experiments had the following composition: 1% yeast extract, 1% enzymatic hydrolysate of soybean meal, 20, 50, 70, 120 or 170 g/l of glucose, the pH was adjusted to 5 with phosphoric acid. 1 ml samples were taken at different time intervals in order to follow glucose consumption and ethanol production. The samples were centrifuged at 10'000 g during 5 minutes, the supernatant was filtered through 0.22 µm pore sizes filters before being stored at -20°C for later HPLC and GC analysis.

3.5 Yeast extract production from *Saccharomyces cerevisiae* fermentation wastes

Many valuable compounds are contained in the waste recovered at the end of an ethanolic fermentation and they can be reused and valorised in a microbial electrolysis cell. To examine this approach enthanol fermentation were realised to obtain good quantities in shake flask at 30°C/180 RPM. The media had

the following composition: 1% enzymatic hydrolysate of soybean meal, 1% of yeast extract and 50 g/l of glucose, the pH was adjusted to 5 with phosphoric acid. The media was inoculated with a *S.cerevisiae* preculture (2 % v/v) before incubation. At the end of the fermentation the totality of the culture was centrifuged at 10'000 g for 15 minutes. 150 g of wet biomass was resuspended in 1 litre of demineralized water. The pH was adjusted to 6 with HCl 35% (Acros Organics, Switzerland), then the suspension was incubated at 50°C for 48 hours/180 RPM. At the end of the incubation the suspension was heated to 80°C for 30 minutes[6]. The cell membranes and other insoluble wastes were eliminated by centrifugation, 10'000 g during 15 minutes. Finally the water was eliminated by evaporation in a Heidolph VV2000 rotary evaporator (Gemini bv, Netherlands) at 40°C under 72 mbar. The powder recovered after evaporation contained the soluble components of the yeast such as: amino acids, peptides, nucleotides and carbohydrates[6].

3.6 *Shewanella oneidensis* MR-1 cultivation

Shewanella oneidensis MR-1 was cultivated according to ATCC recommendations. Before being introduced in the anode compartment of the bioelectrical fuel cell a 100 ml preculture was realised in a 300 ml shake flask at 30°C and 180 RPM. The ATCC medium 18 was used for preculture and experiments. The media had the following composition: 17 g/l Tryptone, 3 g/l of hydrolysate of soybean meal, 2.5 g/l of glucose, 5.0 g/l NaCl, 2.5 g/l K_2HPO_4, the pH was adjusted to 7.3 ± 0.2. When *S. oneidensis* was used in the bioelectrical fuel cell the media was prepared with 20 g/l of glucose instead of 2.5 g/l for the preculture.

The strain was stored on an agar plate in a cold room at 8 °C. The agar plate was prepared by adding 15 g/l of agar to the ATCC medium 18 described above. Every three weeks *S.oneidensis* was transferred on fresh agar plate. The freshly inoculated agar plate was incubated at 30 °C during 48 hours before being stored in the cold room again.

The media were sterilized at 121 °C during 20 minutes before utilisation. Glucose and other constituents were autoclaved separately in order to avoid Maillard reactions and added after sterilization under a laminar air flow cabinet.

3.7 Catholyte composition in microbial fuel cell mode

When the reactor was used as a microbial fuel cell, meaning that an electrical current was generated, the catholyte consisted of a 0.5 M potassium ferricyanide (Sigma-Aldrich, Switzerland) solution buffered at pH 7 by a phosphate buffer 0.1 M containing Na_2HPO_4 and NaH_2PO_4 (Fluka chemika, Switzerland).

3.8 Catholyte composition in microbial electrolysis cell mode

Different catholytes were tested when the bioelectric fuel cell was operated as a microbial electrolysis cell. A phosphate buffer 0.1 M prepared with Na_2HPO_4 and NaH_2PO_4 pH 7. An acidic catholyte was also tested, it consisted of 2 M HCl (Acros Organics, Switzerland) and a more diluted variant of 0.5 M HCl. For experiments without a proton exchange membrane between the anodic and cathodic compartments the anode and cathode were both immersed in the same cultivation medium.

3.9 Bioelectric fuel cell characterization in microbial fuel cell mode

3.9.1 EMF monitoring

The open circuit voltage is the maximum voltage measurable for a microbial fuel cell (infinite resistance, zero current). It represents the difference between the anode and cathode individual potential[7].

$$EMF = E_{cat} - E_{an} \quad (4)$$

EMF: electromotive force [V]

E_{cat}: cathode potential [V]

E_{an}: anode potential [V]

The half-cell oxidation reaction of glucose will theoretically determine the anode potential as for the cathode chamber the theoretical potential is determined by potassium ferricyanide. If one assumes a complete oxidation of glucose at the anode, the theoretical maximum open circuit voltage of the whole MFC is 0.791 V under standards conditions (pH 7 / 298 K)[7].

The media and the catholyte used in this experiment are described in section 3.3 and 3.7. The voltage was recorded by a digital multimeter VC 960 (Voltcraft, Germany). The anode compartment was connected to the COM jack of the multimeter, while the cathode compartment was connected to the VΩ jack. The value of the tension was recorded at a 2 minute interval for 24 hours.

3.9.2 Polarization and power density curves

The anode and cathode compartments were interconnected through a resistance decade box at the end of the open circuit voltage monitoring when a plateau was reached (see section 3.9.1). A multimeter, connected in parallel on a resistance decade box R-Dekade SE 40 (Shärer Elektronic AG, Switzerland) was used to monitor the voltage. 20 resistances were applied decreasingly to the system: 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 8.0, 6.0, 4.0, 2.0, 1.0, 0.9, 0.7, 0.5, 0.3 and 0.1 kΩ. For each resistance the corresponding voltage was recorded. The resistances were changed every 60 seconds.

The power and the current were normalized in view of different characteristics of the reactor, namely the volume of the anode, the true surface area of the anode[8] and the area of the opposite side of the

anode. The experiments were repeated in triplicate and the results given with a confidence interval of 95%. The current, the power and the internal resistance were been calculated as it follows.

The current was calculated using Ohm's law[8]:

$$I = \frac{E}{R_{ext}} \quad (5)$$

I: current [A]

E: tension [V]

R_{ext}: external resistance [Ω]

The power was determined from tensions and currents using the following relation[9]

$$P = E * I = \frac{E^2}{R_{ext}} \quad (6)$$

P: power [W]

I: current [A]

E: tension [V]

R_{ext}: external resistance [Ω]

The power density curves were realised based on the following equation[9]

$$P_{dens} = f(I_{dens}) \quad (7)$$

P_{dens}: power density [mW/m^3] and [mW/m^2]

I_{dens}: current density [mA/m^3] and [mA/m^2]

The power density as a function of the current density is a quadratic equation having the following form: $y = ax^2 + bx + c$. The equation corresponding to the experimental data was determined by polynomial regression. Then the top of the curve was determined knowing that it possess the following coordinates: $\left(\frac{-b}{2a}; \frac{4ac-b^2}{4a}\right)$

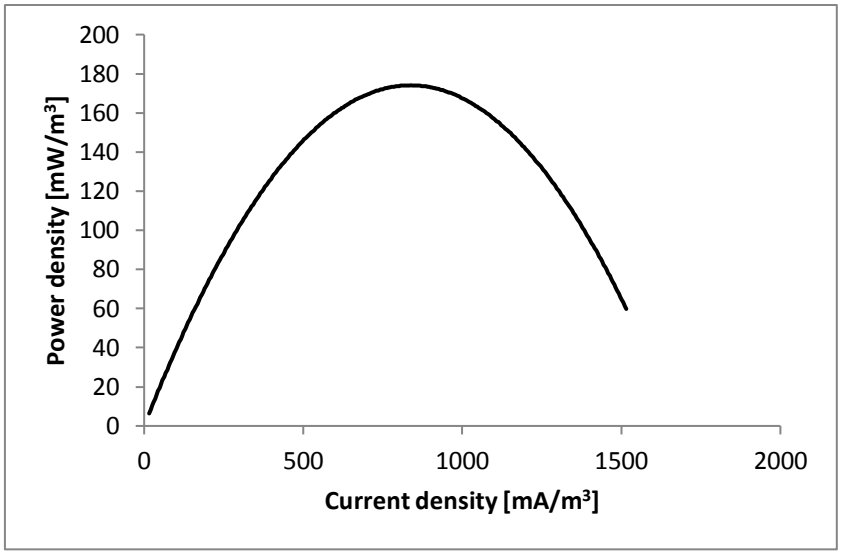

Figure 8: Example of a power density curve, the top of the curve corresponds to the maximum power output.

The polarization curves were realised based on the following equation[9]

$$E = f(I_{dens}) \quad (8)$$

E: tension [V]

I_{dens}: current density [mA/m^3] and [mA/m^2]

The internal resistance is the sum of the resistances encountered by the electron flow through the external circuit, the anode, the cathode and the electrolytes in both anodic and cathodic chambers. The internal resistance includes also the resistance encountered by the proton flow through the membrane between the anodic and cathodic compartments[9, 10]. R_{int} is a useful parameter since the maximum power output is generated when the external resistance equals the sum of the internal resistance of the fuel cell[11, 12].

The internal resistance of the system has been determined with the slope of the polarization curve[10]

$$R_{int} = -\frac{\Delta E}{\Delta I} \quad (9)$$

R_{int}: Internal resistance [Ω]

E: tension [V]

I: current [A]

3.10 Microbial fuel cell operation

Before and after each batch the whole reactor including the tubing was disinfected in a 1% Korsolex (Bode Chemie, Germany) solution overnight.

The yeast cultivation was realised in the anode compartment according to section 3.3 separated from the cathode compartment by a Nafion® proton exchange membrane N117. The cathodic compartment contained a 0.5M potassium ferricyanide catholyte buffered at pH 7 according to section 3.7. Different glucose concentrations were tested, namely 20, 50, 70, 120 and 170 g/l. Each concentration was tested with and without mediator addition. The mediator Methylene Blue was added to achieve a final concentration of 0.3 mM.

The anodic and cathodic compartments were connected through a 1000 Ω external resistance. The COM jack of the multimeter was connected on the anode connection on the external resistance. The VΩ jack of the multimeter was connected on the cathode connection on the external resistance. The multimeter monitored the voltage on the resistance by recording a value every 2 minutes. Then the current was calculated using Ohm's law (see equation 5).

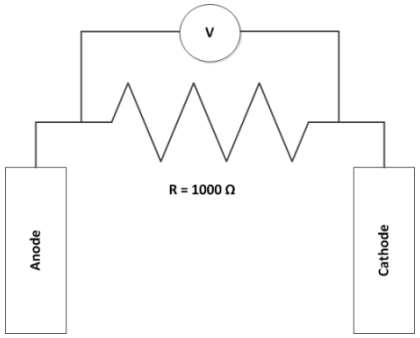

Figure 9: Electrical diagram for current production and monitoring in microbial fuel cell mode.

The number of coulomb produced was calculated by integrating the current over time using trapezoidal rule. The region under the curve was segmented into multiple trapezoids, one between each value recorded by the multimeter (every 2 minutes). The sum of the areas of the trapezoids corresponds to the area under the curve. The area of one trapezoid was calculated as follow:

$$A = \left(\frac{f(x_2)+f(x_1)}{2}\right) * (x_2 - x_1) \qquad (10)$$

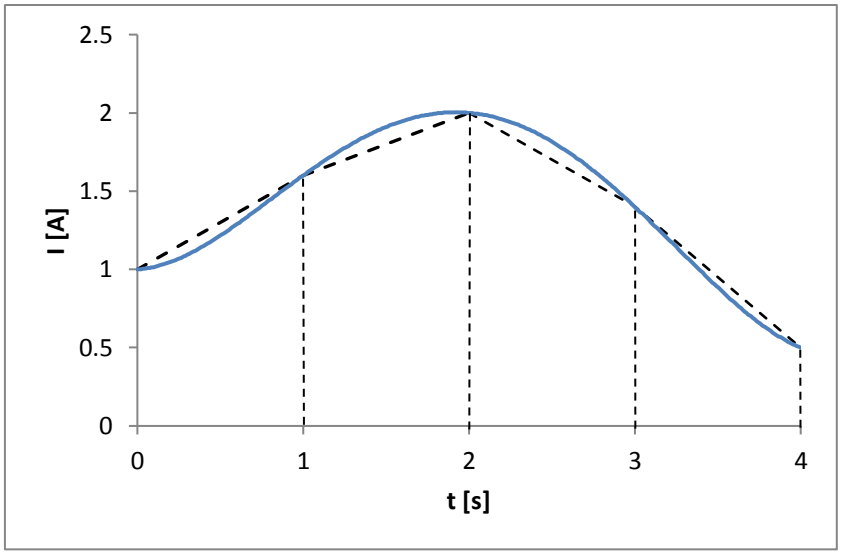

Figure 10: Representation of the trapezoidal rule integration method. The region under the curve was segmented into multiple trapezoids.

$$C_{tot.} = \int_0^t I\, dt \qquad (11)$$

C_{tot}: total of coulombs generated [C]

I: current [A]

t: time [s]

The coulombic efficiency represents the ratio between the number of coulomb recovered and the number of coulomb present in the substrate consumed. The coulombic efficiency[7] has been calculated as follow:

$$C_E = \frac{M_s \int_0^t I\, dt}{F\, b_{es}\, v_{an}\, \Delta c} \qquad (12)$$

C_E: coulombic efficiency [-]

M_s: molecular weight of substrate [g/l]

I: current [A]

t: time [s]

F: Faraday's constant [C/mole e-]

b_{es} : mole of electrons per mole of substrate oxidized [mole e-/mole substrate]

v_{an}: volume of liquid in the anode compartment [l]

Δc: substrate concentration change over batch cycle [g/l]

Samples were taken in regular time intervals in order to follow the glucose consumption and bioethanol production. The samples were centrifuged at 10'000 g for 5 minutes, the supernatant was then filtered

through 0.22 µm pore size filter before stored at -20C°. The samples were analysed according to sections 3.14.1 and 3.14.2.

3.11 Microbial electrolysis cell operation

Before and after each batch the whole reactor including the tubing was disinfected in a 1% Korsolex (Bode Chemie, Germany) solution overnight. *S.cerevisiae* and *S.oneidensis* cultivations were realised according to section 3.3 and 3.6.

When the bioelectrical fuel cell was operated in microbial electrolysis cell mode the nickel cathode was recovered by platinized reticulated vitreous carbon electrodes in order to enhance hydrogen production. The platination was performed according to section 3.2.

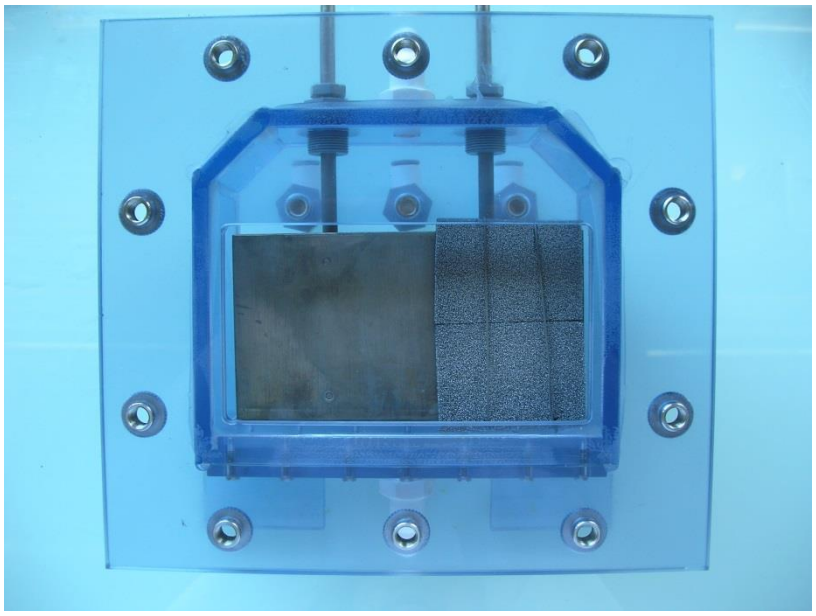

Photo 3: Nickel cathode partially covered by platinized RVC electrodes (on the right). When the bioelectrical fuel cell was operated as a microbial electrolysis cell, the nickel cathode was completely covered with this type of electrodes.

The cultivations were realised in the anodic compartment separated from the cathodic compartment by a Nafion® N117 proton exchange membrane. The catholyte consisted of a phosphate buffer 0.1 M pH 7 (Na_2HPO_4, NaH_2PO_4) or a 0.5 M (also 2 M) HCl (Acros organics, Switzerland) solution (see section 3.8). The anode was connected to the positive lead of a power supply (Voltcraft, Germany), the negative lead was connected over an 11 Ω external resistance to the cathode. The voltage over the resistance was recorded at two minute intervals with a multimeter. The COM jack of the multimeter was connected on the anode connection on the external resistance. The VΩ jack of the multimeter was connected on the cathode connection on the external resistance. The current was calculated using ohm's law (see equation 5).

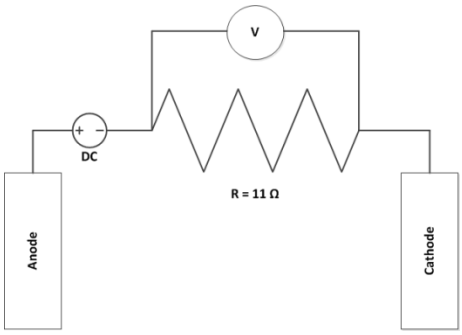

Figure 11: Electrical diagram for current and hydrogen production in microbial electrolysis cell mode.

Coulombic efficiencies were calculated as described in section 3.10 (see equation 12). The energy added to the system by the power supply[5] was determined by the following equation:

$$W_E = \sum_i^n (I_i E_{ps} \Delta t - I_i^2 R_{ext}\, \Delta t) \qquad (13)$$

W_E: energy added [J]

I: current [A]

E_{ps}: power source applied voltage [V]

t: time [s]

R_{ext}: external resistance [Ω]

Once the anodic and cathodic compartments were assembled, connected and filled with their respective solutions the quick connection on the top of the cathodic compartment was connected with polyurethane 5.5 mm diameter gas tight tubing to a milligas-counter in order to monitor released gas volumes. The oxygen present in the catholyte was removed by sparging nitrogen gas 4.5 (≥ 99.995% purity) through the quick connection located at the bottom of the cathode compartment. Realising this operation directly in the set-up allowed removing the oxygen present in the cathode headspace, in the gas tight tubing and the milligas-counter.

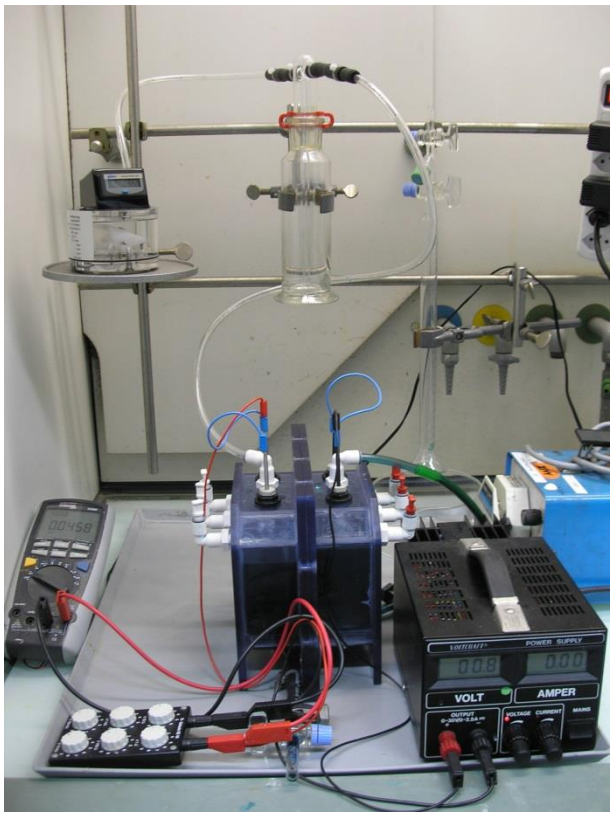

Photo 4: Set-up of the bioelectric fuel cell used in microbial electrolysis cell mode. A voltage was applied to the fuel cell by the power supply on the right, the gas produced was monitored by a milligas-counter placed above the reactor and the current was monitored by a multimeter (on the left) connected over an external resistance.

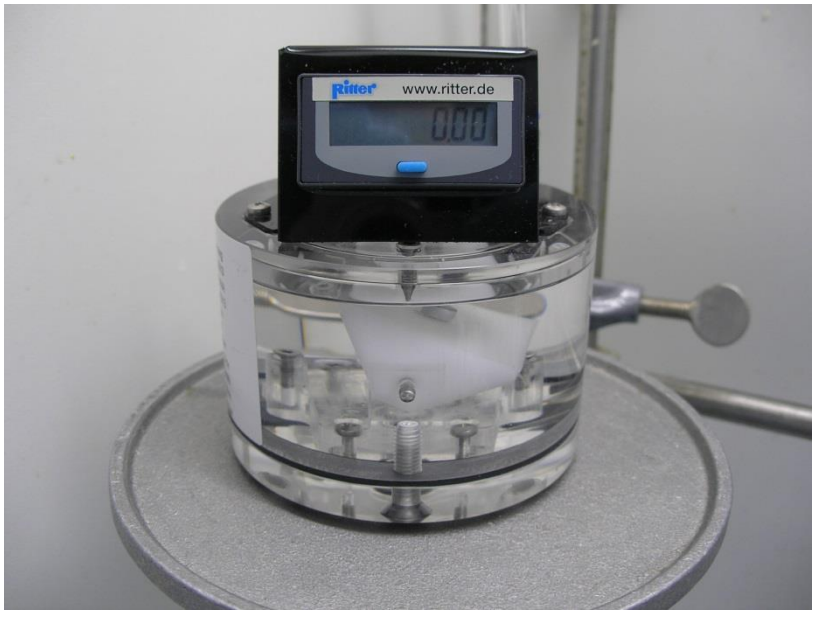

Photo 5: MGC-1 V3.2 PMMA Milligas-counter filled with silicone oil to monitor gas volumes produced in the cathode compartment.

Samples were taken in regular intervals from the anodic compartment in order to follow the glucose consumption and bioethanol production. The samples were centrifuged at 10'000 g during 5 minutes, the supernatant was then filtered through 0.22 µm pore size filters before stored at -20C° in Eppendorf vials. The liquid samples were assayed according to sections 3.14.1 and 3.14.2. Gas samples were taken through a folding skirt stopper (VWR, Switzerland) connected on the gas tight tubing before the milligas-counter (see photo 6). 20ml gas samples were taken with a gas tight syringe model 1025 (Hamilton, USA). The gas samples have been analysed according to section 3.14.3.

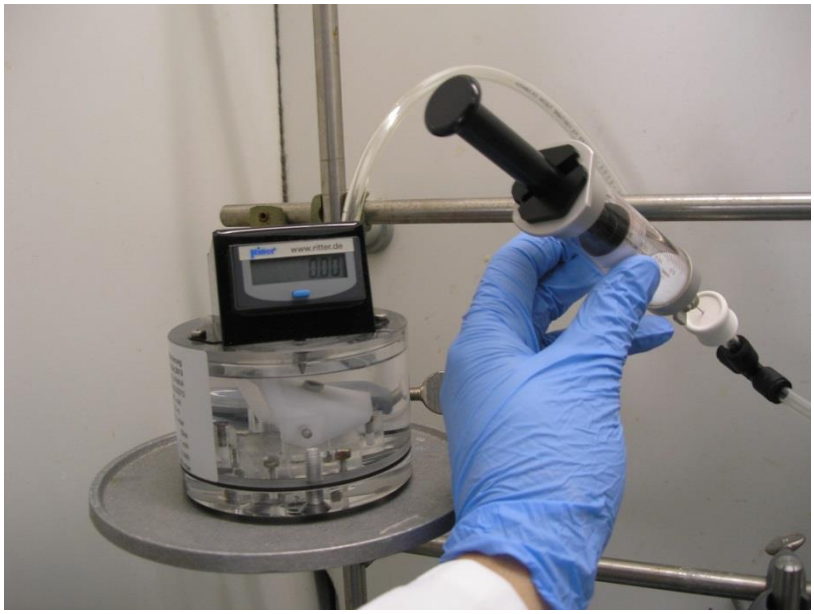

Photo 6: Withdraw of a gas sample through a folding skirt stopper on the gas tight tubing connecting the milligas-counter and the cathodic compartment. The gas samples were taken with a gas tight syringe.

3.12 Fermentation of waste from ethanol production by *Shewanella oneidensis*

A cultivation of *shewanella oneidensis* MR-1 was realized in the anode compartment according to section 3.6. The bioelectric fuel cell was operated as microbial electrolysis cell according to section 3.11. The anode compartment was feed with ATCC medium 18 (see section 3.6) when a drop of current was observed (under 0.1 V). Once a hydrogen production in the cathode compartment was noticed, the content of the anode compartment was filled with 450 ml of *S. cerevisiae* fermentation waste prepared according to section 3.5. For the entire experiment the catholyte consisted of a 0.1M phosphate buffer adjusted to pH 7.

3.13 Methane production from ethanolic fermentation wastes

3.13.1 Production of fermentation wastes for methanisation

Fermentations were realized in 2 litters shake flasks filled with 1.2 l of the following media: 1% yeast extract; 1% soybean meal, 50 g/l glucose, the pH was adjusted to 5.0 with phosphoric acid (Acros Organics, Switzerland). The shake flasks were incubated at 30°C over 48 hours.

During the fermentation 2 ml samples were withdrawn, centrifuged at 10'000g for 10 minutes in a 5415 C microcentrifuge (Eppendorf, Switzerland). The supernatants were filtered through 0.22 µm PTFE filters (Alys-technologies, Switzerland) and stored at -20 °C until analysis. Glucose consumption and ethanol production were determined for each fermentation. Glucose was assayed by HPLC on an Agilent 1100 Series (Agilent, USA) and ethanol was assayed on a Hewlett Packard 6890N GC (Agilent, USA).

At the end of the fermentation the biomass was recovered by centrifugation (10'000g, 10 minutes) in a Hicen XL ultra-centrifuge (Herolab, Germany). Supernatants were poured off and biomass was transferred in sterile Corning Centristar tubes (Sigma-Aldrich, Switzerland). Recovered biomass was then lyophilized in a telstar cryodos freeze drier (Swiss vacuum technologies SA, Switzerland) at -80°C under 0.250 mbar over 72 hours.

3.13.2 Reactor set-up and operation for electromethanisation

The wastes recovered from *S. cerevisiae* fermentation were digested in an up-scale reactor designed for electromethanogenesis. The reactor was a 492x280x298 mm (Lxlxh) tank containing 10 anodes made of RVC 280x18x130 mm (Lxlxh) (ERG aerospace, USA) and 9 cathodes made of nickel 190x2x130 mm (Lxlxh) (Bibus metals, Switzerland). The electrodes were immersed in the bulk in an alternative way, meaning that each cathode was surrounded by anodes.

The 10 anodes were connected to each other to the positive lead of a power supply (Voltcraft, Germany). The negative lead of the power supply was then connected to a 10 Ω resistor. The other side of the resistor was connected to the cathodes.

The reactor was then filled with 11.5 litters of either activated sludge or fermentation wastes. The headspace was sparged with nitrogen 4.5 over 30 minutes (Pangas, Switzerland) in order to remove oxygen. Gas composition of the headspace was monitored by gas sampling through a folding skirt stopper (VWR, Switzerland) with gas tight syringe (Hamilton, USA). Gas samples were assayed on a micro-GC 490 (Agilent, USA). The tension was monitored on the external resistor using a digital multimeter VC 960 (Voltcraft, Germany).

3.13.3 Reactor Acclimation for electromethanisation

A biofilm was formed on the electrodes using activated sewage sludge originated from the first fermenter of the wastewater treatment plant of Châteauneuf (Ville de Sion). The reactor was filled with 11.5 litre of activated sewage sludge, a tension of 0.75V was applied between the electrodes. The reactor was operated under these conditions over 20 days. Both gas composition and current production were monitored over the acclimation phase.

3.13.4 Fermentation waste digestion and bio-electromethanisation

Once the reactor was acclimated the digestion of the fermentation waste was realized. 23g of fermentation waste was dissolved in 11.5 l of distilled water. The activated sludge was removed from the reactor and replaced by dissolved fermentation waste. Three applied potentials were used, 0.0 V, 0.75V and 1.5V. Each run was realized with its own fermentation waste solution and an acclimation phase. The digestion was conducted over 36 days.

Gas samples were taken in order to determine gas composition of the headspace and current was monitored by measuring the voltage over an external resistance. The chemical oxygen demand (COD) was determined at the beginning and the end of the experiment in order to evaluate the amount of organic matter removed during the process.

3.13.5 Fermentation waste digestion with in-situ H_2 production

An acclimation phase was realized as described in section 3.13.3. The activated sludge was then removed and replaced by 23g of fermentation wastes dissolved in 14 L distilled water. As previously described 10 anodes made of RVC were used. For in situ H_2 production a single cathode was immersed in the bulk. The nickel cathode was covered by platinized carbon cloth (see section 3.2), equipped with Nafion® proton exchange membrane and filled with 0.1M sterile phosphate buffer pH 7.0. A potential of 1.5V was applied over 27 days.

Gas was sampled from the reactor headspace and analysed by micro-GC according to section 3.14.3. Voltage was recorded over a 10Ω external resistance and current was calculated using Ohm's law. Liquid samples were taken at the beginning and the end of the digestion to determine the COD consumption over the process (see section 3.14.4).

3.14 Analytic

3.14.1 Glucose analysis

Glucose concentrations were assayed by HPLC using an Agilent 1100 Series equipped with an Aminex HPX-87H column (Bio-Rad, USA). The temperature of the column was set to 35°C, 10 µl samples were injected for each analysis, a 5 mM sulphuric acid (Acros organics, Switzerland) solution was used as mobile phase at a flow rate of 0.600 ml/min under 90 bar. The detection was performed with a G1362A RID detector (Agilent, USA). A calibration curve was realised for each run, the following standards were prepared: 0.1 g/l; 0.5 g/l; 1 g/l; 2 g/l; 3 g/l glucose (Merk, Switzerland).

3.14.2 Ethanol analysis

Ethanol concentrations were assayed by solid phase micro extraction gas chromatography using a Hewlett Packard 6890N GC apparatus mounted with a HP-5MS column (Agilent, USA). The samples

were incubated for 5 minutes at 40°C before being extracted for 30 minutes. The injection was realised at 250 °C for 3 minutes. A 0.01% n-butanol (Fluka, Switzerland) internal standard was added to the ethanol standards and the samples. A calibration curve was realised for each run, the following ethanol standards were prepared: 0.01%; 0.1%; 0.5%; 1%; 2% (Fluka, Switzerland). The calibration curves were realised by plotting the ethanol/n-butanol area ratios against the standards concentrations. A FID detector was used for the assay.

3.14.3 Gas analysis

The gas produced was sampled in the reactor headspace with a gas tight syringe (Hamilton, USA), 20 ml were taken. Samples were then injected in a micro-GC 490 (Agilent; USA) mounted with 2 columns, a MS5A for the analysis of H_2, O_2, N_2, CH_4 and a PPU column for the analysis of CO_2 and CH_4. The first column operated at 80.0°C at 200 kPa, the second column was operated at the same temperature at 150 kPa.

3.14.4 Chemical oxygen demand

5 ml of the following solution: 0.04 mol/l $K_2Cr_2O_7$ (Sigma Aldrich, Switzerland), 80.0 g/l $HgSO_4$ (Fluka Analytical, Swizerland), 1.80 mol/l H_2SO_4 (Acros Organics, Swizerland) was added to 15 ml of a solution containing 10.0 g/l Ag_2SO_4 (Fischer Chemical, Switzerland) and 17.4 mol/l H_2SO_4. Finally 10ml of 10 times diluted sample was added. The mixture was heated at 148 °C over 2 hours. The solution was then cooled down and 50ml of distilled water was added. A blank was realized using 10ml of distilled water instead of 10ml of diluted sample.

The excess of $K_2Cr_2O_7$ was then determined by titration. Two drops of ferroin indicator (Sigma-Aldrich, Switzerland) were added to the solution, the titration was realized with the following solution: 47.0 g/l $(NH_4)_2Fe(SO_4)_2$ $6H_2O$ (Sigma Aldrich, Swizerland), 0.360 mol/l H_2SO_4. The solution should turn from green-blue to orange-braun.

The chemical oxygen demand was calculated as follow:

$$\frac{8000C(V1-V2)}{V0} \qquad (14)$$

8000: masse de ½ O_2 [mg/l]
C : Concentration of the titrant [mole/l]
V1: Volume of titrant solution used for the blanck [ml]
V2: Volume of titrant solution used for the sample [ml]
V0: Volume of the sample [ml]

4. Results and Discussion

4.1 Bioelectric fuel cell characterization in microbial fuel cell mode

4.1.1 Open circuit voltage

The open circuit voltage is characterized by two half reactions. The first one represents the glucose oxidation by *S. cerevisiae* in the anode compartment, while the other one represents potassium ferricyanide reduction in the cathode compartment.

Anode half reaction

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \qquad E_{anode}=-0.43V^{7} \qquad (14)$$

Cathode half reaction

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \qquad E_{cathode}=0.361V^{7} \qquad (15)$$

Based on equation 4 and if one assumes that the reactions occurs at pH 7, 298K and that the concentrations of $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ are the same in the cathode compartment, the theoretical maximum electromotive force (emf) is 0.791V.

In practice the measured voltage increased until it reached a plateau after about 15 hours of cultivation (See Fig.12). The mean maximum emf for the three runs was 0.427 ± 0.020 [V].

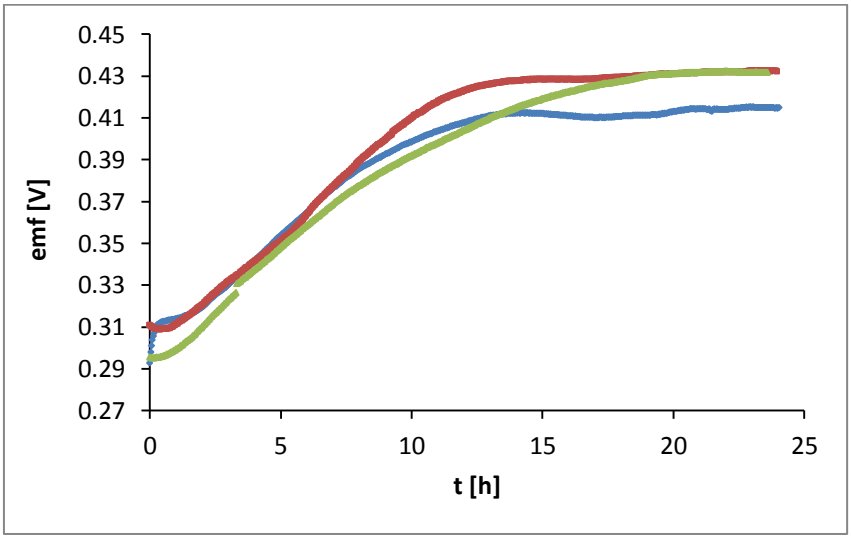

Figure 12: Overall cell electromotive force (emf) recorded during the cultivation.

4.1.2 Internal resistance

In order to characterize the performance of the MFC, polarization experiments were conducted. A series of external resistances was applied to the fuel cell, the voltage was recorded and the current calculated using Ohm's law (see equation 5). The relation between the cell voltage and the current was expressed by the following function[10]:

$$\boldsymbol{E_{cell} = OCV - IR_{int}} \qquad (16)$$

E_{cell}: cell voltage [V]

OCV: open circuit voltage [V]

I: current [A]

R_{int}: internal resistance [Ω]

Based on the polarization curves (See fig. 13), it was determined that the microbial fuel cell used in this experiment had an average internal resistance of 954.3 ± 210.6 [Ω] when operated with RVC anode and nickel cathode.

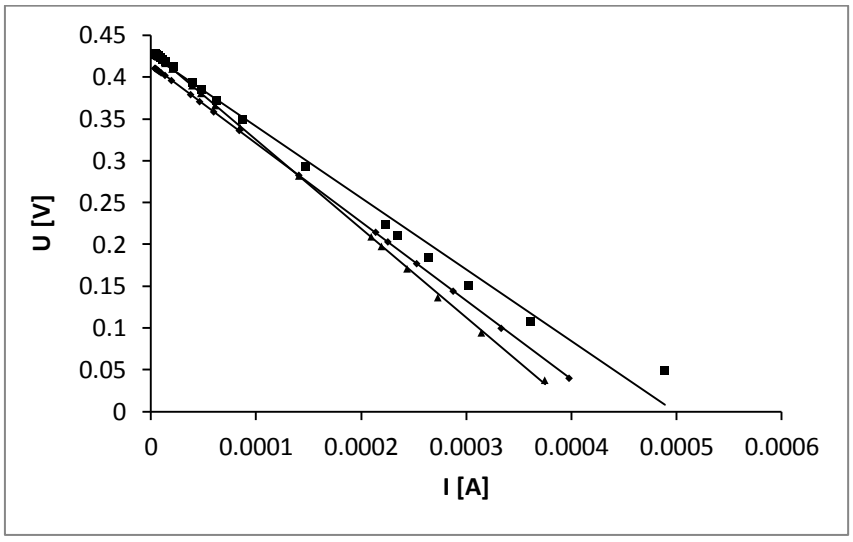

Figure 13: Polarization curves measured from three distinctive *S. cerevisiae* cultivations and renewed catholyte.

4.1.3 Power density

The mean maximum power output calculated from power density curves (See fig. 13) was 175.8 ± 71.8 [mW/m^3] normalized to the anode volume, 0.027 ± 0.0038 [mW/m^2] normalized to the true surface area of the anode and 2.54 ± 0.35 [mW/m^2] normalized to the opposite surface area of the anode.

As mentioned above the optimal performance of the microbial fuel cell was observed when the internal and external resistances were identical. In the present case the fuel cell showed an internal resistance of 954.3 ± 210.6 [Ω] for a maximum power output under an external resistance of 899.4 ± 435.1 [Ω]. For further experiments in which the reactor was operated in microbial fuel cell mode the external resistance was set to 1000 [Ω] in order to recover the maximum efficiency achievable by the fuel cell.

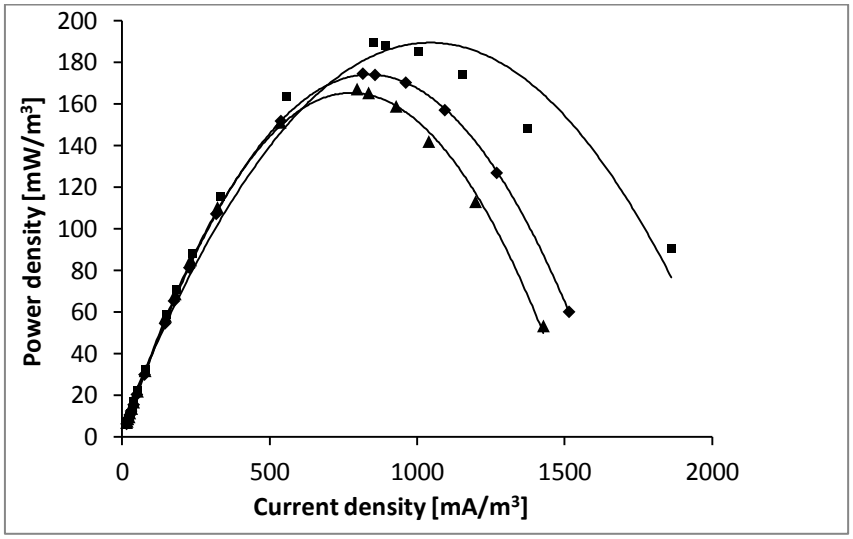

Figure 14: Power density curves normalized to the anode volume. The power densities were measured from three distinctive *S. cerevisiae* cultivations and renewed catholyte.

The details of the data regarding the characterization of the bioelectric fuel cell in microbial fuel cell mode and the power density curves normalized to the anode true surface area and anode opposite surface area are presented in the annex 8.1.

4.2 Current and bioethanol co-production

4.2.1 Current generation in absence of yeast

In order to validate the fact, that recorded currents originated from the oxidative metabolism of *S.cerevisiae*, blank experiments were performed in complete absence of microorganisms in the anode compartment. The bioelectrical fuel cell was operated as a microbial fuel cell during 48 hours. The anode compartment was filled with the following media: 10 g/l yeast extract, 10 g/l enzymatic hydrolysate of soybean meal and 170 g/l of glucose. The pH was adjusted to 5 with phosphoric acid. The experiment was realised with a mediator, 0.3 mM Methylene Blue in processed anolyte and without mediator.

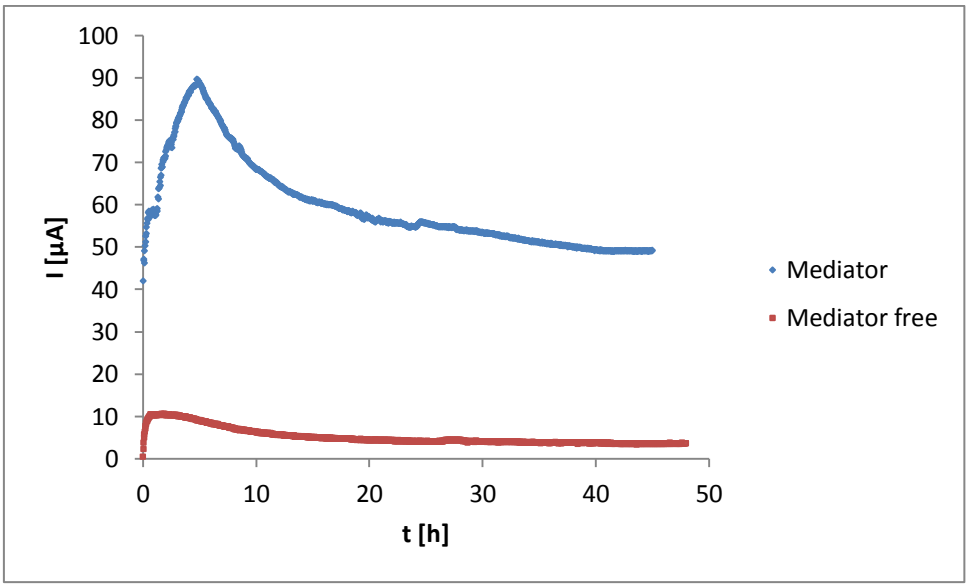

Figure 15: Current monitored using medium containing a mediator and mediator free medium. The anode compartment was not inoculated by *S. cerevisiae* or any other microorganism. The anodic and cathodic compartments were separated by a proton exchange membrane.

A current was observed even if no biocatalyst was present in the anode compartment. This current originated from the oxidation of electroactive compounds contained in the medium, especially in the yeast extract and enzymatic hydrolysate of soybean meal[13]. When no mediator was added to the media an average background current of 4.7 µA was observed throughout the experiment. When 0.3 mM of Methylene Blue was added a higher background current was generated, with an average of 59.5 µA and a peak at 89.7 µA at the beginning of the experiment. This higher current was due to the mediator properties of Methylene Blue. Methylene Blue is easily reduced and oxidized, the consequence is a faster and more complete oxidation of the medium leading to higher current density.

4.2.2 Ethanol generation as blank experiment in shake flasks

Five fermentations were realised in 200 ml shake flasks without any current generation. These experiments were realised in order to evaluate the potential impact of recovering an electrical current

from an *S. cerevisiae* ethanolic fermentation. The medium used for these fermentations was the same as used in the bioelectrical fuel cell when an electrical current was produced in absence of a mediator. Five different glucose concentrations were tested, namely 170, 120, 70, 50 and 20 g/l. The ethanol production yields were calculated based on the theoretical production of 0.51 g ethanol per gram of hexose (see equation 3).

Table 1: Ethanol control experiment fermentations realised in shake flasks. No mediator was present and no electrical current generated. The final ethanol concentration was determined for 200 ml volume. The ethanol production yield was calculated based on the theoretical ethanol production of 0.51 g ethanol/g glucose. (B1-B5= blank 1-blank 5).

Entry	Initial glucose concentration [g/l]	Glucose consumed [g]	Final ethanol concentration [% v/v]	Ethanol production yield [%]
B1	170	34.1	13.5	122.3
B2	120	23.2	8.81	117.5
B3	70	15.7	5.34	105.3
B4	50	9.85	3.00	94.1
B5	20	3.87	1.14	93.8

The highest glucose concentrations leaded to the highest ethanol yields. In fact a high glucose concentration will generate a pyruvate overflow and a saturation of the respiratory metabolic pathway. Furthermore, the cultivations have not been aerated creating an environment with low oxygen content. These two conditions will stimulate the fermentative metabolic pathway of *S. cerevisiae*[2] leading to high ethanol productivity. As mentioned above the ethanol yields were calculated based on glucose concentration, but under high fermentation metabolic pathway stimulation, ethanol yields higher than 100% can be achieved since yeast extract and hydrolysate of soybean meal used in the media (see section 3.3) contains other fermentable substrates[6, 15].

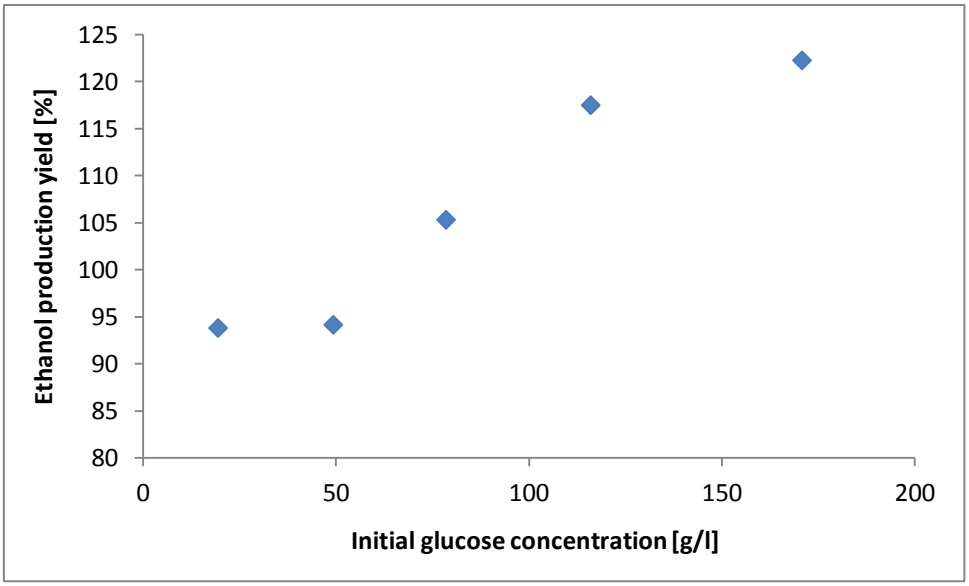

Figure 16: Ethanol production yield as a function of glucose concentration for the fermentations realised in shake flasks. Higher glucose concentrations favours the conversion of glucose into ethanol.

4.2.3 Current and ethanol co-production with *S. cerevisiae* in absence of a mediator

By operating the bioelectrical fuel cell as microbial fuel cell an electrical current was recovered from anodic fermentation. The anodic and cathodic compartments were separated by a proton exchange membrane. The cathode contained a 0.5M potassium ferricyanide solution as final electron acceptor, essential to generate an electron flow. The anodic and cathodic compartments were connected together through a 1000 Ohm external resistance. This resistance was chosen since it corresponds to the resistance presenting the best performance in terms of power output and was close to the internal resistance of the system used in microbial fuel cell mode (see section 4.1.3).

The glucose concentrations tested were identical to the ones used for blank experiments realised in shake flasks, 170, 120, 70, 50 and 20 g/l. The media had also the same composition: 1% yeast extract and 1% enzymatic hydrolysate of soybean meal adjusted to pH 5 with phosphoric acid. For this series of experiments no mediator was added.

For each batch the glucose and ethanol respective concentrations were followed while the currents were recorded. Ethanol yields were calculated based on the theoretical production of 0.51 g ethanol/g of glucose (see equation 3). The number of coulombs recovered were calculated by integrating the current over time (see equation 11). The results are presented in the following table.

Table 2: Microbial fuel cell use with *S. cerevisiae* in the absence of a mediator. The final ethanol concentration was determined from 450 ml volume. The ethanol yield was calculated based on the theoretical ethanol production of 0.51 g ethanol/g glucose. (NM1-NM5 = No mediator 1-No mediator 5).

Entry	Initial glucose concentration [g/l]	Batch Duration [h]	Glucose consumed [g]	Final ethanol concentration [% v/v]	Ethanol production yield [%]	Coulomb generated [C]
NM1	170	48	71.8	3.37	32.7	15.4
NM2	120	48	46.9	4.30	63.8	7.27
NM3	70	48	31.6	3.00	66.2	11.0
NM4	50	48	23.4	2.48	73.7	10.4
NM5	20	24	8.22	0.95	80.1	5.88

The tendency in Table 2 shows that a higher glucose concentration results in a more important generation of coulombs for the same volume. In fact, in theory 24 moles of electrons can potentially be released per mole of glucose consumed when completely converted into carbon dioxide (see equation 1), then if more glucose is available more electrons will be released and transferred to the anode. Another observation concerned the ethanol yields. For each glucose concentration tested the ethanol yields are smaller than the ones achieved in shake flasks under the same conditions but without any current generation. Meaning that generating an electrical current from the fermentation will decrease ethanol productivity at the expense of the electrical current. It is also interesting to notice that the ethanol yields follow the opposite tendency than the one realised in shake flasks. When an electrical current is recovered from the fermentation an increase of the glucose concentration does not increase the ethanol yield.

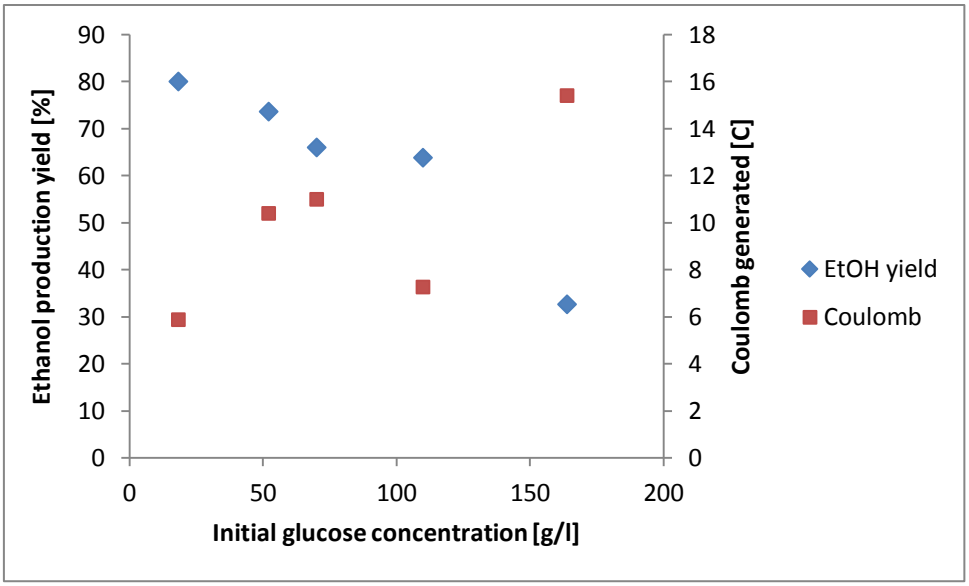

Figure 17: Ethanol yields and number of coulombs generated as a function of initial glucose concentration for fermentations realised in microbial fuel cell mode in absence of a mediator.

The experiments realised in microbial fuel cell mode in the absence of a biocatalyst in the anodic compartment demonstrated that the current originated from the oxidative metabolism of *S. cerevisiae*. The following figure illustrates the current generated over time with different glucose concentrations in comparison to currents generated in the absence of yeast.

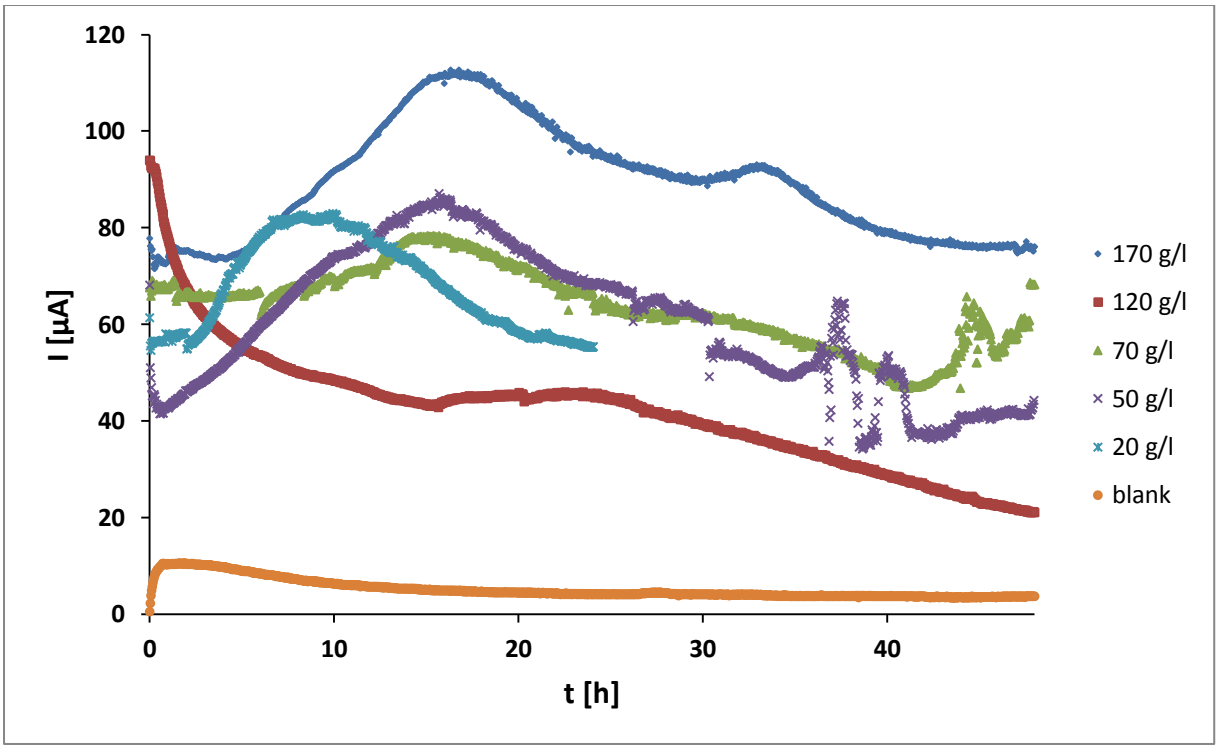

Figure 18: Currents recorded over time at different glucose concentration when the bioelectrical fuel cell was operated as microbial fuel cell without mediator. Blank = media oxidation without *S. cerevisiae*.

Coulombic efficiencies were determined as the ratio between the number of coulombs generated and the number of coulomb generated if glucose was entirely converted into electrical current (see equation 12).

Table 3: Coulombic efficiencies for the microbial fuel cell operated with *S. cerevisiae* in absence of mediator.

Entry	Initial glucose concentration [g/l]	Batch duration [h]	Glucose consumed [g]	Coulombs present in substrate consumed [C]	Coulombs generated [C]	Coulombic efficiency [%]
NM1	170	48	71.8	$9.23*10^5$	15.4	0.0016
NM2	120	48	46.9	$6.03*10^5$	7.27	0.0012
NM3	70	48	31.6	$4.06*10^5$	11.0	0.0027
NM4	50	48	23.4	$3.00*10^5$	10.4	0.0035
NM5	20	24	8.22	$1.06*10^5$	5.88	0.0055

4.2.4 Current and ethanol co-production with *S. cerevisiae* and Methylene Blue as mediator

The bioelectrical fuel cell operated in microbial fuel cell mode was repeated using *S. cerevisiae* as biocatalyst in the anodic compartment with the same glucose concentrations used for fermentations realised in shake flasks and without mediator, 170, 120, 70, 50 and 20 g/l. A proton exchange membrane was inserted between the anodic and cathodic compartments. The compartments were connected over an external circuit with a 1000 Ohm external resistance and the catholyte consisted of a 0.5M potassium ferricyanide solution. The only difference between this series of experiments and the one reported in section 4.2.3 is the addition of a mediator. In fact, in order to enhance the electron transfer between *S. cerevisiae* and the anode Methylene Blue was added in addition of the yeast extract, the enzymatic hydrolysate of soybean meal and the glucose to a final concentration of 0.3mM.

Throughout the fermentation samples were taken in order to follow glucose consumption and ethanol production. The current was recorded over time in order to calculate the number of coulomb generated. The results are reported in the following table for batches containing 0.3 mM of Methylene Blue as mediator.

Table 4: Microbial fuel cell with *S. cerevisiae* in presence of 0.3mM Methylene Blue as mediator. The final ethanol concentration was determined on 450 ml volume. The ethanol production yield was calculated based on the theoretical ethanol production of 0.51 g ethanol/g glucose. (C1-C5 = Current 1-Current 5).

Entry	Initial glucose concentration [g/l]	Batch duration [h]	Glucose consumed [g]	Final ethanol concentration [%]	Ethanol production yield [%]	Coulomb generated [C]
C1	170	54	75.6	5.49	50.6	23.3
C2	120	48	51.5	6.32	85.3	15.6
C3	70	48	31.6	1.94	42.7	12.9
C4	50	48	22.5	1.54	47.5	16.7
C5	20	24	10.7	0.95	61.7	10.3

The fact that the electrons and then the electrical current originated from the microorganisms was confirmed by comparison with a blank experiment, in which the media was oxidized in the absence of any microorganism. For each glucose concentration tested the current was higher when *S. cerevisiae* acted as biocatalyst in the anodic compartment.

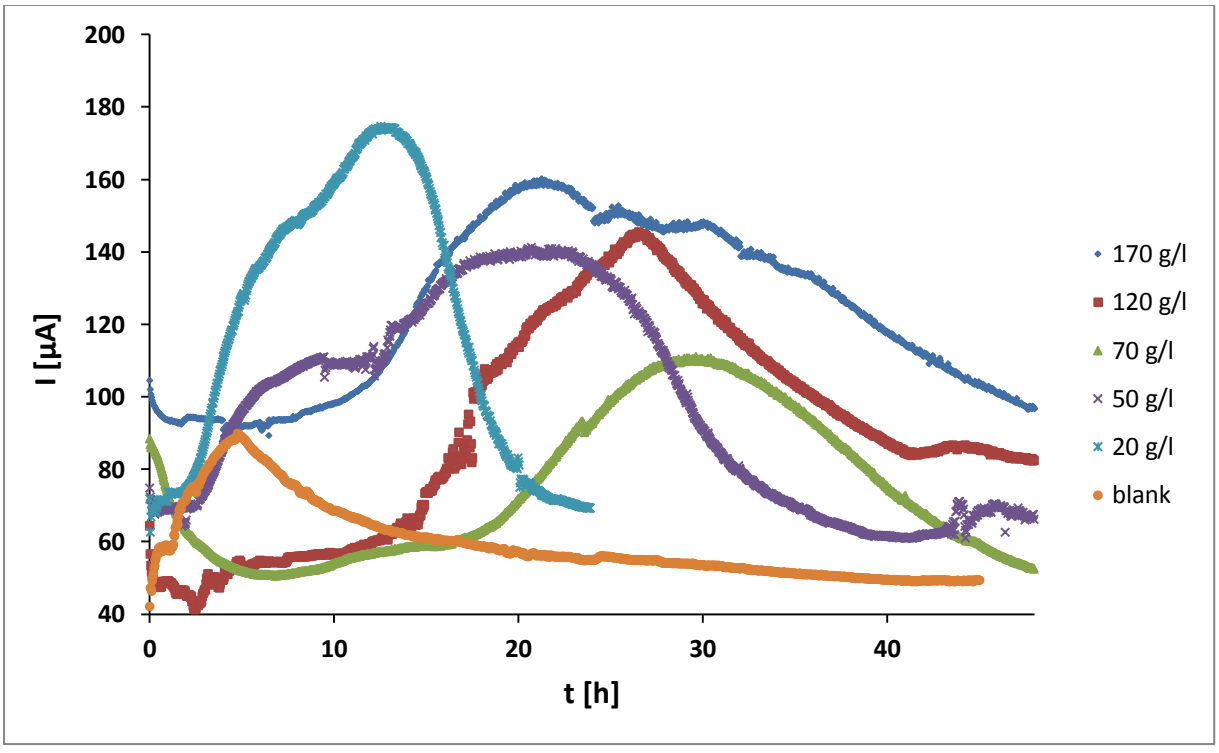

Figure 19: Currents recorded over time with different glucose concentration when the bioelectrical fuel cell was operated as microbial fuel cell with 0.3 mM of Methylene Blue as mediator. Blank = media oxidation without *S. cerevisiae*.

In comparison with the results obtained in the absence of a mediator the number of coulomb generated

was higher for each glucose concentration tested. The mediator acted as an electron shuttle between the biocatalysts and the anode. After being reduced by the electrons released by the biocatalysts metabolism, the Methylene Blue will be oxidized on the anode, enhancing the electron recovery. Higher glucose concentrations generated more coulombs. In fact, when more substrate is degraded more electrons are released and transferred to the anode. The following figure illustrates the number of coulomb generated over 48 hours (24 hours for the glucose concentration of 20 g/l) for the different glucose concentrations with and without Methylene Blue.

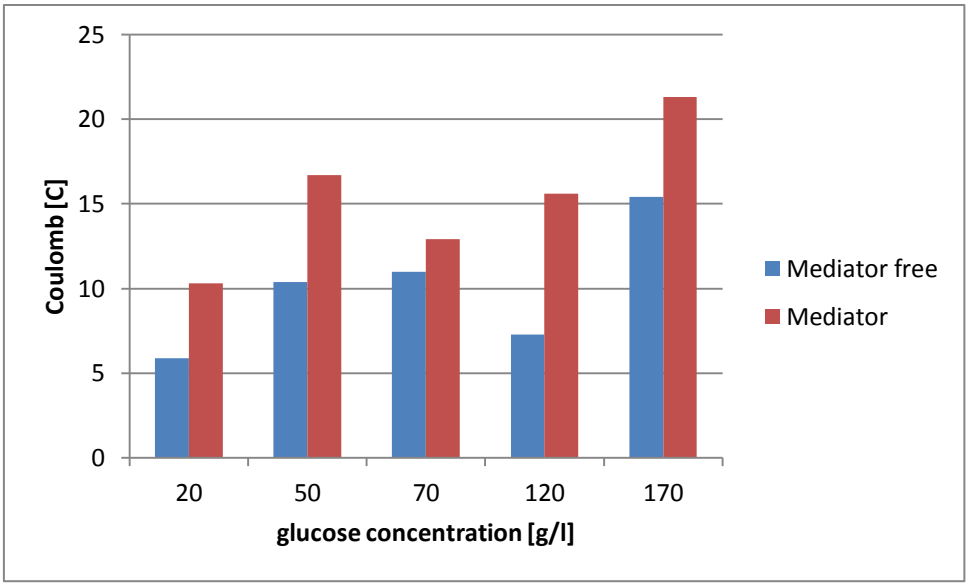

Figure 20: Number of coulomb generated with different initial glucose concentration in presence and absence of a mediator.

None of the experiments in which an electrical current was generated reached ethanol yields as high as the one obtained with shake flasks. For the smaller glucose concentrations, 20, 50 and 70 g/l the experiments realised with Methylene Blue as mediator presents smaller ethanol yield than experiments realised without a mediator. For the higher glucose concentrations, meaning 120 and 170 g/l this difference was no longer observed.

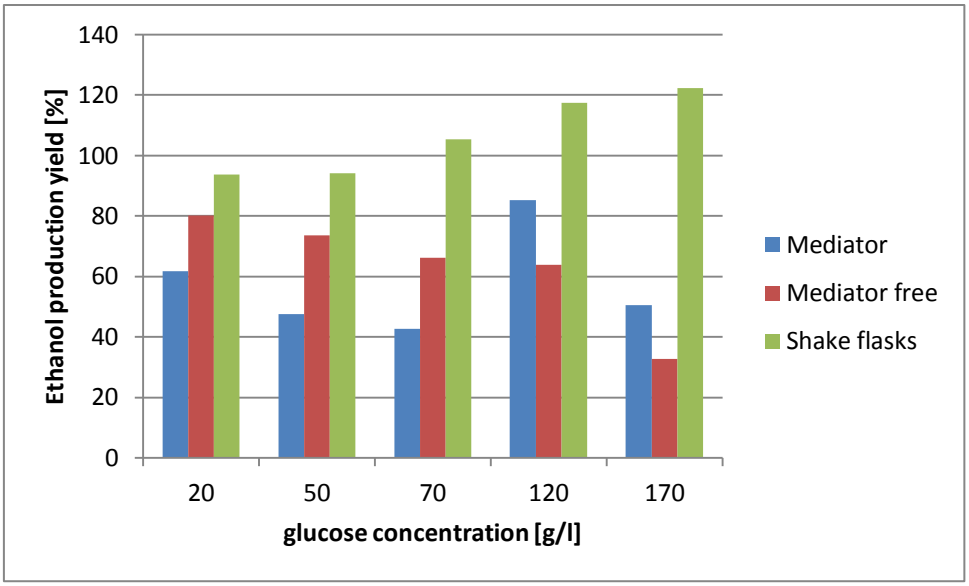

Figure 21: Ethanol production yields at different glucose concentrations for the fermentations realised in shake flasks, in absence of mediator (microbial fuel cell mode) and in presence of mediator (microbial fuel cell mode).

Coulombic efficiencies were calculated according to equation 12. It represents the ratio between the number of coulombs potentially recovered if we assume that 24 moles of electrons were released per mole of glucose consumed and the coulombs actually recovered.

Table 5: Coulomb efficiencies for the microbial fuel cell, operated with *S. cerevisiae* and 0.3 mM of Methylene Blue as mediator. Coulomb efficiencies were calculated based on total glucose consumption.

Entry	Initial glucose concentration [g/l]	Batch Duration [h]	Glucose consumed [g]	Coulombs present in substrate consumed 10^5 [C]	Coulombs generated [C]	Coulombic efficiency [%]
C1	170	54	75.6	9.83	23.3	0.0024
C2	120	48	51.5	6.62	15.6	0.0024
C3	70	48	31.6	5.41	12.9	0.0024
C4	50	48	22.5	2.90	16.7	0.0058
C5	20	24	10.7	1.53	10.3	0.0067

4.3 Hydrogen and bioethanol co-production

4.3.1 Microbial electrolysis cell operated without membrane

For this experiment the membrane separating anode and cathode was removed. By removing the membrane the internal resistance of the system decreased, increasing electron and proton fluxes[16]. Both the anode and cathode electrodes were immersed in the *S. cerevisiae* fermentation. The cathode was equipped with platinised RVC electrodes as described in section 3.11. The reactor was operated with an initial glucose concentration of 170 g/l and 0.3 mM of Methylene Blue was added as mediator. A voltage of 1.3 V was applied after overnight acclimatization. The reactor was connected with gas tight tubing to a milligas-counter in order to determine produced gas volume over time. The glucose consumption and the ethanol production were monitored as well as the gas composition.

After 16 hours of acclimation 1.3 V was applied to the system. Over the 32 hours under the applied voltage, 101.4 coulombs were produced and 22.4 l of gas was generated at a flow of 0.59 l/h. The voltage applied corresponds to an energy added to the system of 130.8 J. The coulomb efficiency of the system, calculated from the beginning of the applied voltage until the end of the experiment, was 0.013 %.

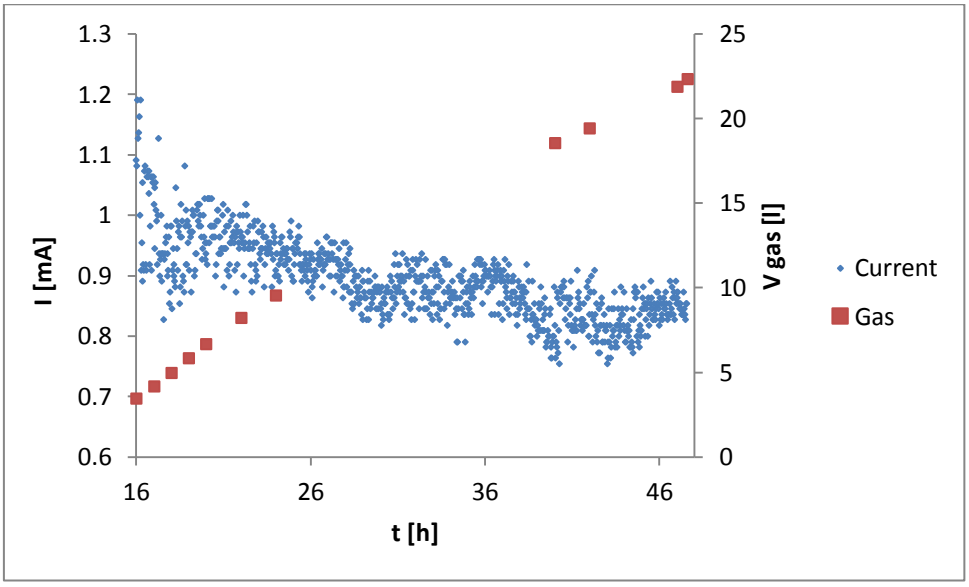

Figure 22: Current and biogas production for the microbial electrolysis cell operated without membrane with 1.3 V of applied voltage.

The gas composition remained quite constant throughout the experiment. The gas produced, mostly carbon dioxide, had the following averaged composition: 88.8 ± 1.2 % of CO_2, 8.8 ± 1 % of N_2, 2.39 ± 0.3 % of O_2. If hydrogen was produced could not be determined by the available detector. In fact the high CO_2 production exercised a diluting effect on the other gas fractions.

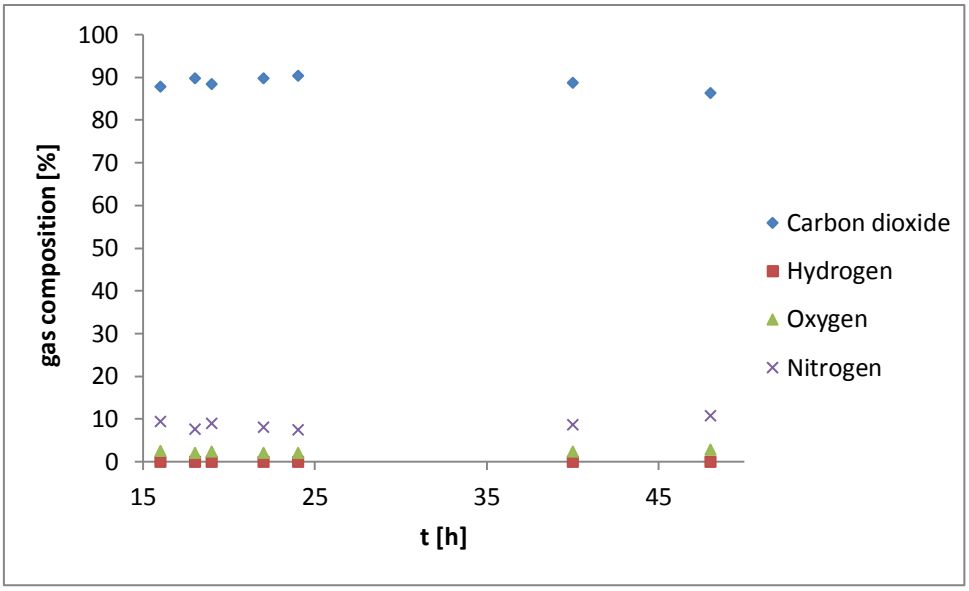

Figure 23: Gas composition in the reactor headspace during the microbial electrolysis cell operation without membrane under 1.3 V of applied voltage.

Glucose fermentation by *S. cerevisiae* produces high carbon dioxide quantities. 2 moles of CO_2 are released per mole of glucose converted into ethanol through the fermentation metabolic pathway of the yeast while 6 moles are released per mole of glucose through the respiration pathway. 104.8 g of glucose were consumed over the batch cycle for an ethanol yield of 91.5 % based on the theoretical production of 0.51g EtOH/g of hexose (see equation 3). The totality of glucose was not consumed over the 48 hours of fermentation (acclimatization and under applied voltage). With the same initial glucose concentration in previous experiments (in microbial fuel cell mode and shake flasks) the glucose was consumed after 48 hours. This suggests that when a voltage is added the glucose consumption rate of the yeast decreased.

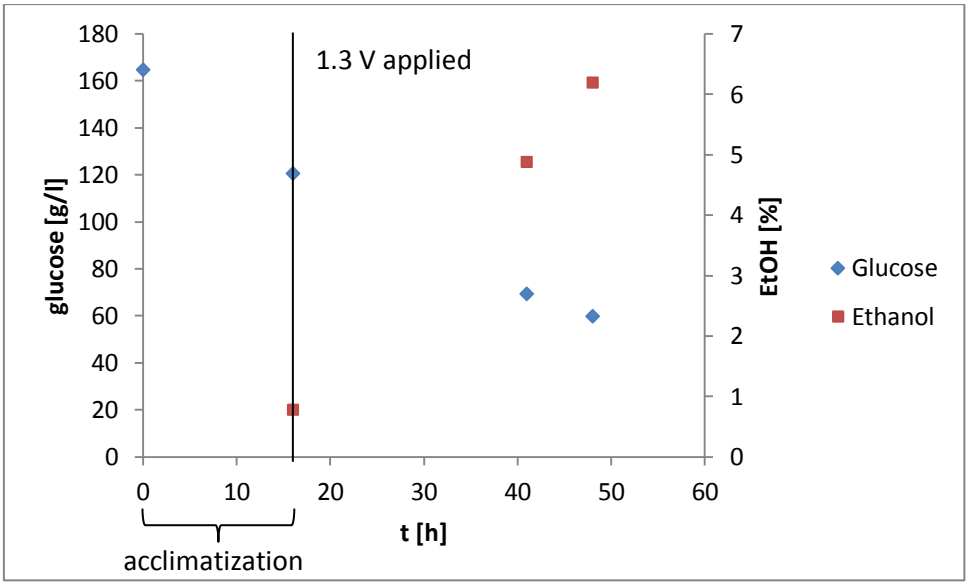

Figure 24: Glucose consumption and ethanol production during the fermentation realised in the microbial electrolysis cell operated without membrane.

4.3.2 Dual chambered Microbial electrolysis cell operated with a phosphate buffer as catholyte

A proton exchange membrane was inserted between the anodic and cathodic compartments in order to separate their respective gas productions. For this experiments the cathodic compartment was filled with a phosphate buffer (pH 7), while the fermentation took place with 0.3 mM of Methylene Blue as mediator and an initial glucose concentration of 170 g/l in the anodic compartment. The cultivation was acclimatized over night before the application of 2.0, 3.0 and 4.0 V to the system this time.

Increasing the applied voltage will increase the electromotive force and more current will be recovered with the fermentation. A higher current density implies an increase of the proton migration through the proton exchange membrane in order to guarantee electro neutrality enhancing the potential for hydrogen production. A higher applied voltage implies higher energy consumption even if the number of coulombs generated increased.

Table 6: Coulomb generated, coulombic efficiency and energy consumed by the power supply. The electrical energy recovered has been subtracted to the energy consumed by the applied voltage and reported has energy added.

U applied [V]	Coulomb generated [C]	Coulombe efficiency [%]	Energy added [J]
2.0	39.2	0.0072	78.2
3.0	142.2	0.026	425.1
4.0	290.8	0.027	867.4

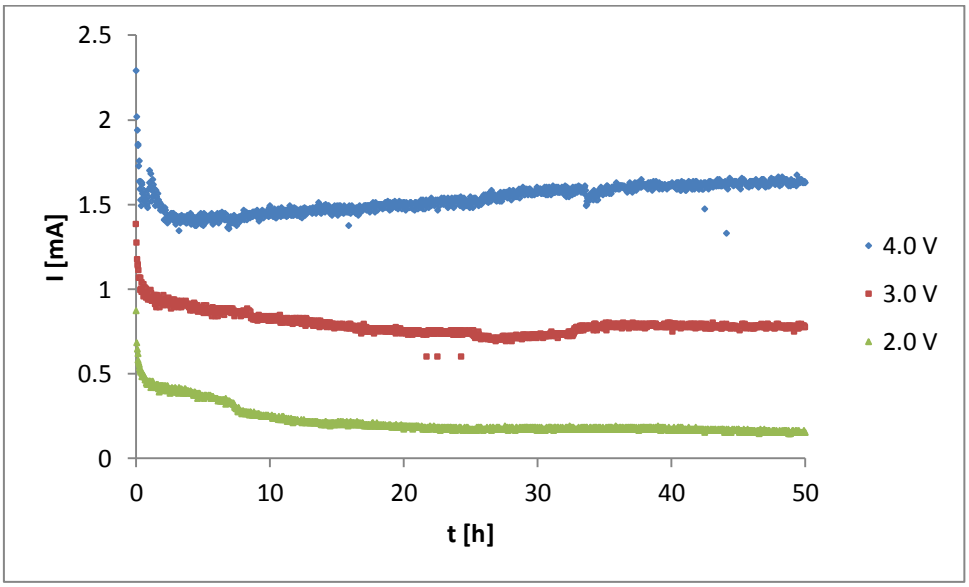

Figure 25: Currents recorded with 2.0, 3.0 and 4.0 V applied in the microbial electrolysis cell with a phosphate buffer as catholyte.

The gas composition of the anodic and cathodic compartments was analysed after 24 and 50 hours using 2.0, 3.0 and 4.0 V of applied voltage. For both experiments the anode compartment headspace contained mostly carbon dioxide originating from yeast fermentation. No hydrogen was produced in the cathodic

compartment applying 2.0 V, while only traces were detectable under 3.0 and 4.0V. CO_2 production was observed in the cathodic compartment. A microscopic analyse of the phosphate buffer at the end of the experiment revealed the presence of a bacillus shaped bacteria contamination.

Table 7: Gas composition in the anodic and cathodic compartments of the microbial electrolysis cell operated with a 0.1M phosphate buffer as catholyte.

		Anode					cathode					
U applied [V]	**T [h]**	**O_2 [%]**	**N_2 [%]**	**CO_2 [%]**	**H_2 [%]**	**CH_4 [%]**	**O_2 [%]**	**N_2 [%]**	**CO_2 [%]**	**H_2 [%]**	**CH_4 [%]**	**EtOH prod. Yield [%]**
2.0	24	1.04	3.56	95.4	0	0	1.56	96.5	1.94	0	0	57.0
	50	1.34	5.07	93.6	0	0	1.77	94.1	4.06	0	0	
3.0	24	0.99	3.44	95.5	0	0	1.93	95.6	2.38	0	0	58.0
	50	1.17	6.20	92.6	0	0	1.75	95.5	2.68	0.002	0	
4.0	24	1.82	7.08	91.1	0	0	1.68	97.7	0.53	0.004	0	54.3
	50	2.54	9.42	88.3	0	0	1.49	94.5	3.93	0.014	0	

The ethanol production yields were close to the one measured with the same initial glucose concentration in microbial fuel cell mode in the presence of a mediator. Under the applied voltage of 2.0 V the ethanol yield reached 57.03 % with 3.0 V 58.0 % and 4.0 V 54.3%. As mentioned before applying a voltage seems to reduce the glucose consumption rate. In fact it took about 72 hours including the acclimation and hydrogen production phase to reach the depletion of the substrate while in previous experiments (shake flasks and microbial fuel cell mode) it was reached in about 48 hours.

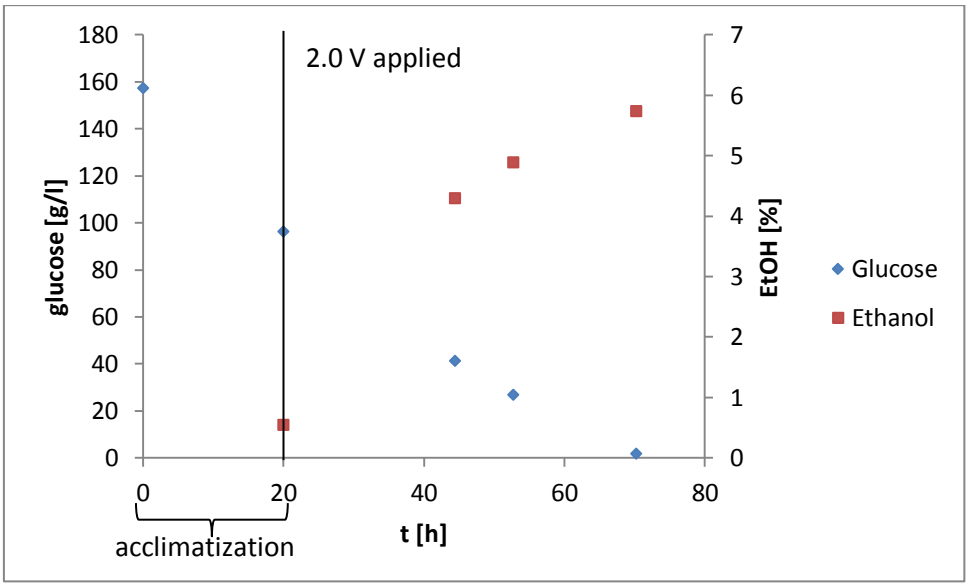

Figure 26: Glucose consumption and ethanol production for the microbial electrolysis cell operated with a phosphate buffer as catholyte under 2.0 V.

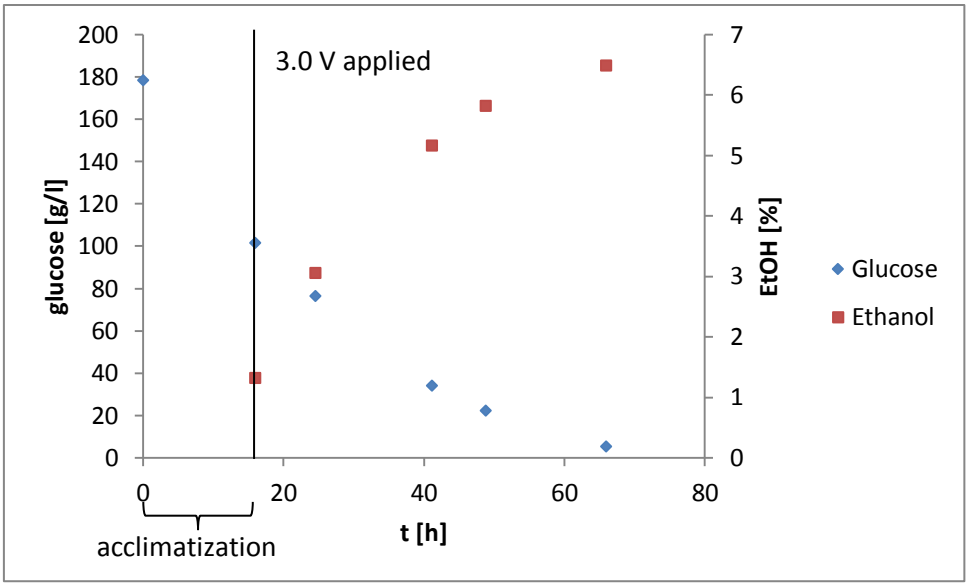

Figure 27: Glucose consumption and ethanol production under microbial electrolysis cell conditions with a phosphate buffer as catholyte and 3.0 V of applied voltage.

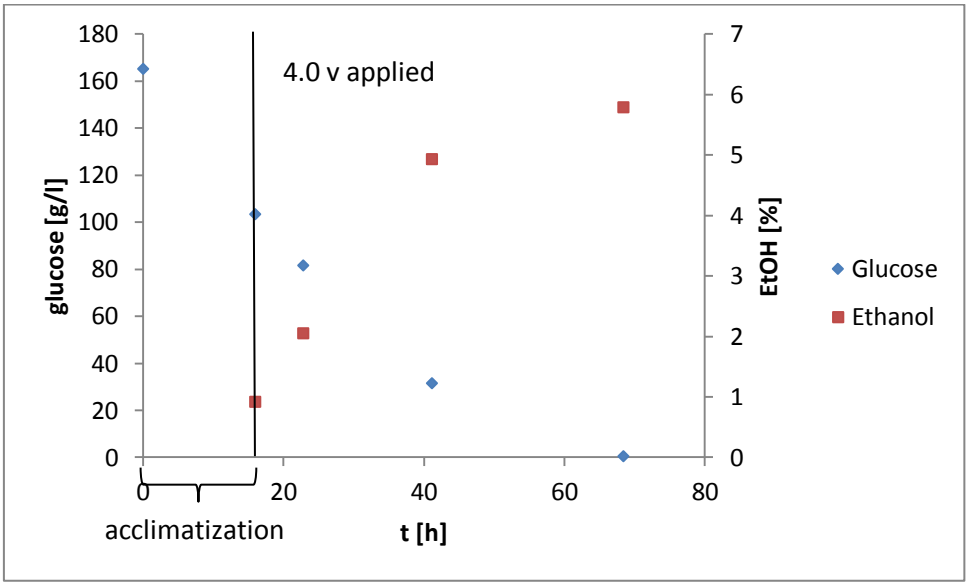

Figure 28: Glucose consumption and ethanol production for the microbial electrolysis cell operated with a phosphate buffer as catholyte with 4.0 V of applied voltage.

The impact of the applied voltage on the number of cells was investigated by counting the cells in a Neubauer chamber from samples taken throughout the process. A drastic decrease of cells per millilitre was observed once 4.0 V was applied to the system. This decrease occurred whereas more than 100 g/l of glucose was still available as substrate.

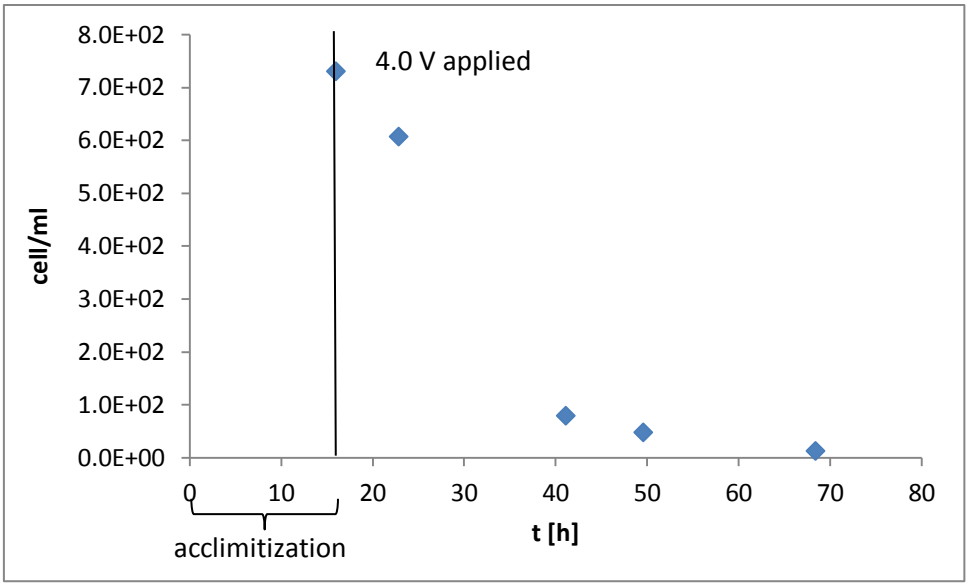

Figure 29: Number of yeast cells per millilitre once 4.0 V was applied.

4.3.3 Dual chambered Microbial electrolysis cell operated with HCl solutions as catholyte

For this series of experiments the phosphate buffer used as catholyte was replaced by an HCl solution. Two different concentrations of HCl were tested, 2.0 M and 0.5 M. The media contained 170 g/l of glucose and 0.3 mM of Methylene Blue as mediator. The cultivation was acclimatized over night before voltage application. The applied voltage can be reduced when HCl was used as catholyte, 1.3 V was applied between the anode and the cathode.

29.2 coulombs were produced over 48 hours of applied voltage when the cathodic compartment was filled with a 0.5 M HCl solution (coulombic efficiency of 0.0042 %) while 13.9 coulombs were produced with the 2.0 M HCl catholyte (coulombic efficiency of 0.0061 %). The external voltage corresponded to an added energy of 37.8 J with 0.5 M HCl as catholyte and 18.1 J with 2 M HCl as catholyte. Regarding the gas production, 0.48 ml of hydrogen was produced with 0.5 M HCl as catholyte and 0.67 ml of hydrogen was recovered with 2.0 M HCl as catholyte. If the hydrogen produced originates from protons liberated by the microorganisms in the anode compartment a higher coulomb production should result with a higher hydrogen production. The fact that this rule is not respected for both experiments suggests that the hydrogen does not originate from the protons released in the anodic compartment but from the catholyte, who should function as storage of protons.

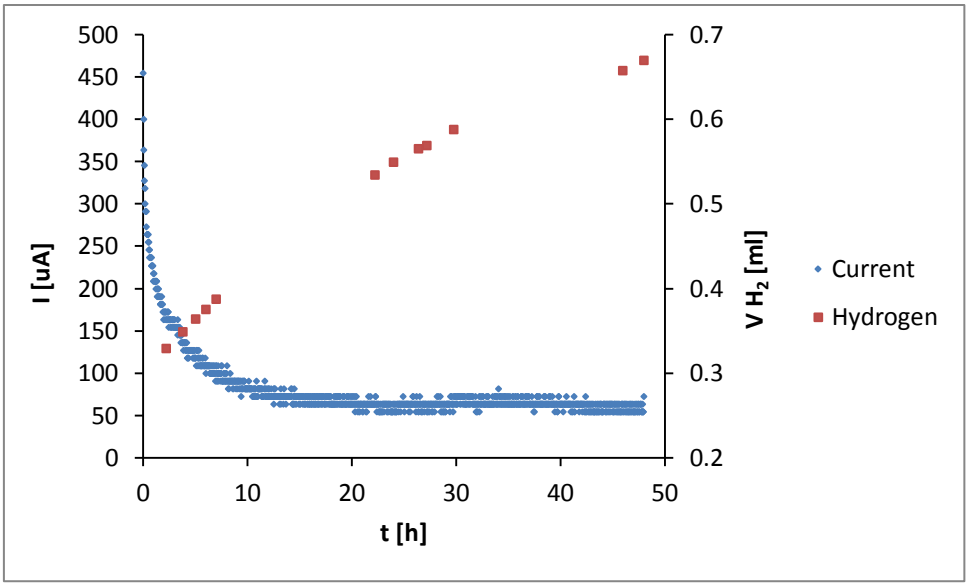

Figure 30: Current and hydrogen production in microbial electrolysis cell operated with a 2.0 M HCl solution as catholyte under 1.3 V of applied voltage, after overnight acclimatization of the fermentation.

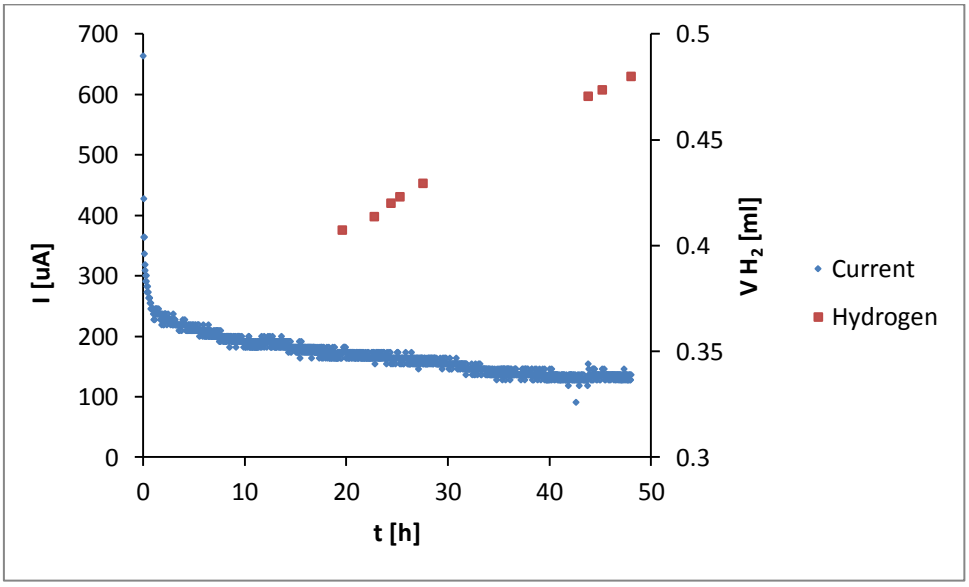

Figure 31: Current and hydrogen production in a microbial electrolysis cell operated with a 0.5 M HCl solution as catholyte and 1.3 V of applied voltage, after overnight acclimatization.

The ethanol yields were quite low for these two experiments, 31.6 % for the 0.5 M HCl catholyte and 10.0 % for the 2.0 M HCl catholyte. This can be explained by the impact of high acid concentration in the cathode. The pH in the anode dropped therefore due to the HCl solutions used as catholyte. In fact the pH in the anodic compartment at the end of the batch realised with 0.5 M HCl as catholyte had a value of 1.9 and the pH at end of the batch realised with 2.0 M HCl as cathoylte had a value of 1.3. Those pH are too low and prevent a good metabolic activity in the anode compartment, this lead to decreasing glucose consumption and consequently to decreasing ethanol productivity.

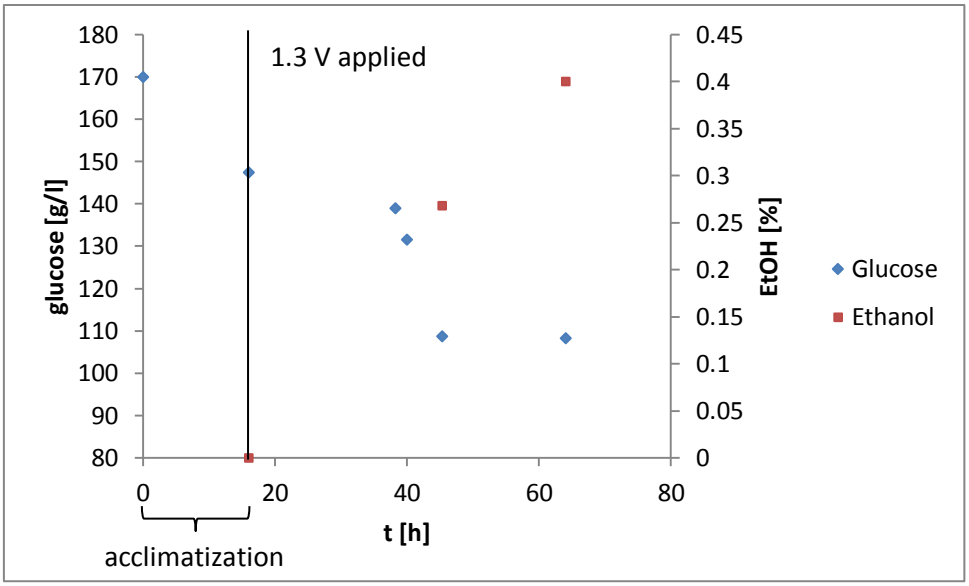

Figure 32: Glucose consumption and ethanol production under microbial electrolysis cell conditions with 2.0 M HCl as catholyte and an applied voltage of 1.3 V.

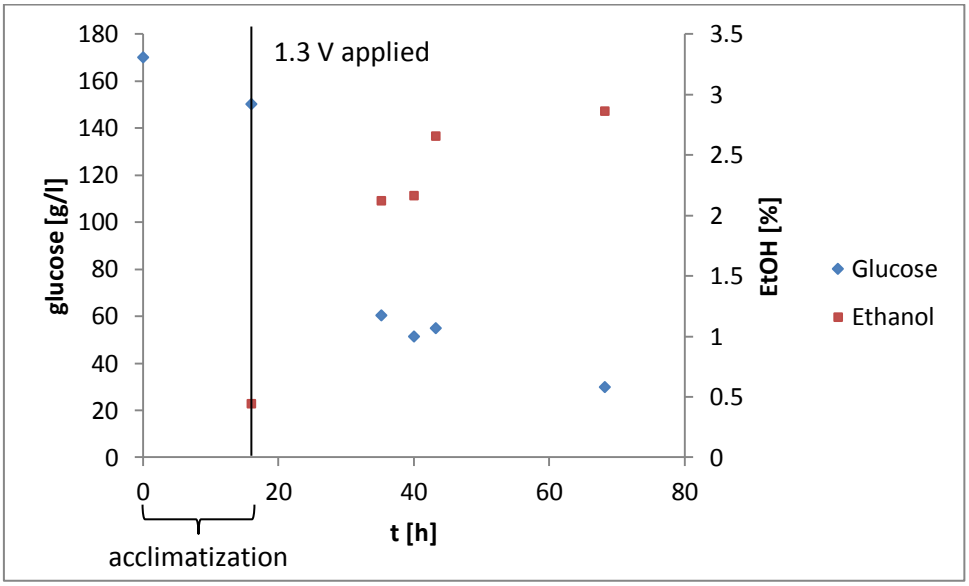

Figure 33: Glucose consumption and ethanol production in microbial electrolysis cell operated with HCl 0.5 M as catholyte under an applied voltage of 1.3 V.

4.4 Hydrogen production using *Shewanella oneidensis* as biocatalyst

A *Shewanella oneidensis* microbial electrolysis cell has been acclimated over 14 days with the aim to digest wastes recovered from an *S. cerevisiae* fermentation realised in shake flasks. The cultivation was realised according to section 3.6. The media in the anode was replaced when a drop of the current was noticed with fresh medium of the same composition except at days 8 and 10 for which the glucose concentration was increased to 40 g/l and day 13 to 70 g/l in order to increase the metabolic activity before waste fermentation. The cathode was filled with phosphate buffer 0.1 M pH 7 and separated from the anode by a proton exchange membrane. The microbial electrolysis cell was operated that way with

1.3 V of applied voltage, the gas composition in the cathode headspace was analysed. 106.7 g of glucose were consumed over the 14 days of acclimatization of the microbial electrolysis cell for a total of 158.8 coulombs generated, resulting in a coulombic efficiency of 0.012 %. The application of 1.3 V over the 14 days of acclimatization corresponds to an energy of 206 J added to the system.

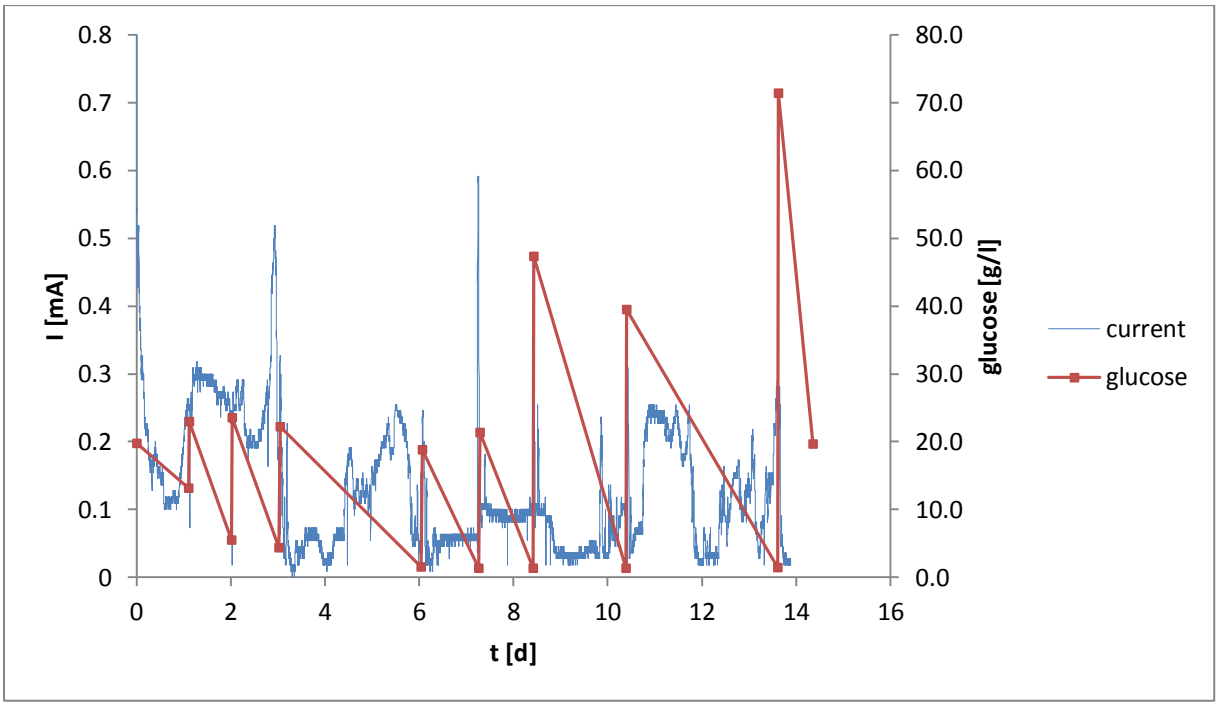

Figure 34: Glucose concentration and current recorded throughout the *S.oneidensis* acclimatization in the microbial electrolysis cell under 1.3 V with phosphate buffer as catholyte.

The gas in the cathode headspace was mainly nitrogen due to oxygen removal at the beginning of the experiment. Traces of hydrogen were detected in the cathode headspace after day 3. A slow increase of hydrogen concentration was noticed from this point. The concentration of carbon dioxide increases during the experiments also after 3 days of operation revealing a contamination of the buffer used as catholyte.

Table 8: Gas composition in the cathode headspace with *S. oneidensis* as biocatalyst in the anode and a phosphate buffer as catholyte under 1.3 V of applied voltage.

t [d]	N_2 [%]	O_2 [%]	CO_2 [%]	H_2 [%]	H_2 [ml]
1	98.4	1.58	0	0	0
2	97.7	2.30	0	0	0
3	96.9	3.02	0.29	0.008	0.020
6	94.7	3.14	2.06	0.045	0.11
7	93.1	3.86	2.98	0.046	0.12
8	92.3	3.59	4.02	0.051	0.13
10	89.6	3.88	6.42	0.066	0.17
14	85.08	3.63	11.2	0.066	0.17

After 14 days of operation, the solution in the anode compartment was replaced by concentrated *S.cerevisiae* fermentation waste. The wastes were prepared according to section 3.5 from 150g of wet biomass recovered from 3 liter fermentation with an ethanol production yield of 81.9 % for an initial glucose concentration of 50 g/l. The microbial electrolysis cell was operated under 1.3 V for 72 hours. 93.9 coulombs were generated once the fermentation wastes have been inserted in the anodic compartment. A gas sample has been taken at the end of the wastes digestion by *S. oneidensis* in the cathode headspace.

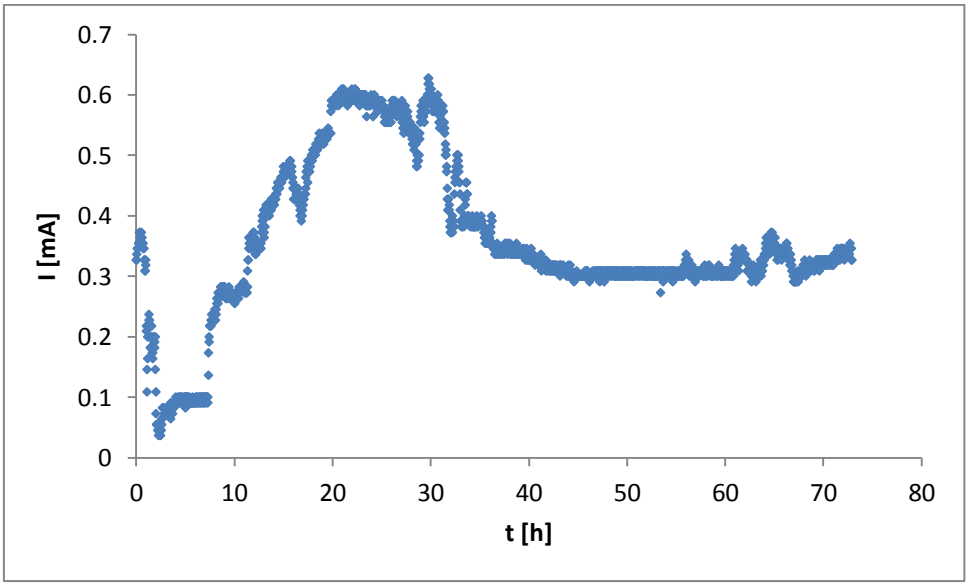

Figure 35: Current recorded over time during the digestion of fermentation waste from ethanol production by *S. oneidensis* under 1.3 V with a phosphate buffer as catholyte.

A small difference can be observed in the gas composition between the sample taken at the end of the *S.oneidensis* acclimatization and at the end of the wastes digestion. This evolution in the gas composition and the curve of the current generated over time suggest that such fermentation wastes can be potentially used as substrate in systems like microbial fuel and electrolysis cell.

Table 9: gas composition at the end of the acclimatization and the end of the wastes digestion in the cathode headspace.

	N_2 [%]	O_2 [%]	CO_2 [%]	H_2 [%]	H_2 [ml]
End of acclimatization	85.08	3.63	11.2	0.066	0.17
End of wastes digestion	81.8	4.34	13.8	0.070	0.18

4.5 Methane production from ethanolic fermentation waste

4.5.1 Methanisation of fermentation wastes, bioelectromethanisation

The first step consisted of the formation of an active biofilm on the electrodes. The reactor was filled with activated sewage sludge withdrawn from the first fermenter of a wastewater treatment plant. Nitrogen was sparged in the headspace of the reactor in order to remove oxygen. 0.75 V were applied between anodes and cathodes in order to enhance the multiplication of exoelectrogenic microorganisms on the electrodes. The gas production over this acclimation phase is presented in the annex 8.5.

In parallel ethanolic fermentations were realized in shake flask. The biomass was collected, dried and resuspended in distilled water (see section 3.13.1). This solution of fermentation waste replaced the activated sewage sludge used for the acclimation phase. The fermentation waste was digested under different applied voltages between the electrodes, respectively 0V, 0.75V and 1.5V.

Three fermentation were realized, the first fermentation provided an ethanol yield of 64.2 ± 11.1 %, the second fermentation 71.9 ± 3.8% and the third fermentation 63.5 ± 11.6%. The wastes recovered from the first fermentation were digested without applied voltage, the wastes recovered from the second fermentation were digested under 0.75V and the wastes recovered from the last fermentation were digested under 1.5V.

The gas composition in the headspace of the reactor was sampled on a daily basis and analysed according to section 3.14.3. The methane and carbon dioxide production are presented in the following figures.

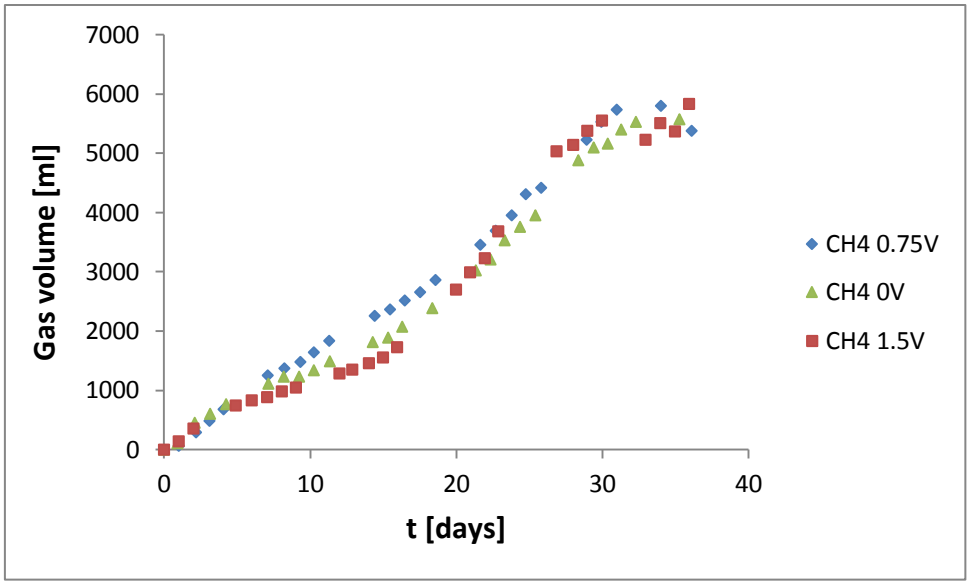

Figure 36: Methane production during the digestion of fermentation wastes under different applied voltage.

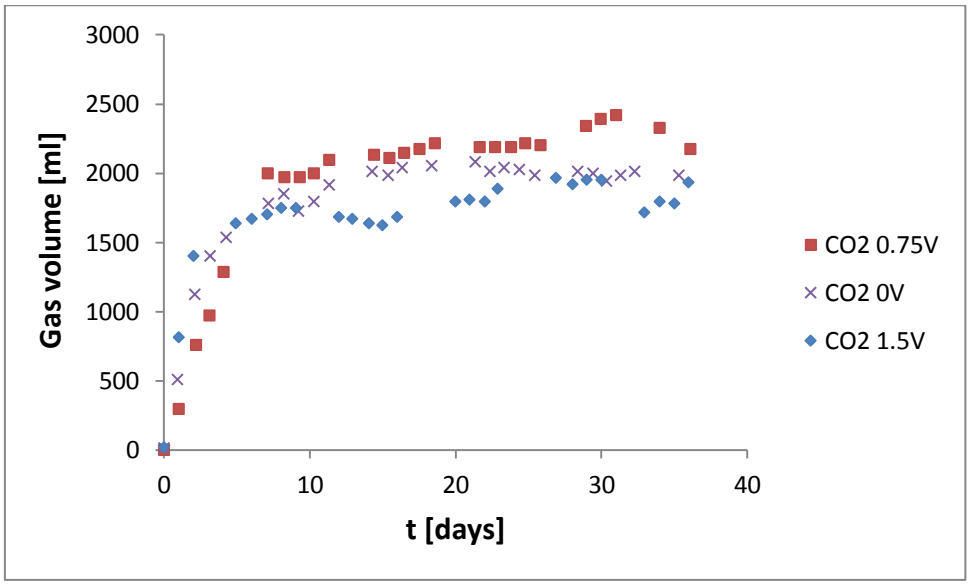

Figure 37 Carbon dioxyde production during the digestion of fermentation waste under different applied voltage.

The chemical oxygen demand was determined according to section 3.14.4 at the beginning and the end of the digestion in order to evaluate the quantity of organic matter removed over the process. The portion of organic matter converted into methane was also calculated, the results are presented in the table 10.

The fraction of chemical oxygen demand removed over each batch was similar (see table 10), but the COD conversion yield into methane increased with the applied voltage especially at 1.50V where 91.1 % of the COD removed was converted into methane against 62.7% under 0.75V and 61.3% when no voltage was applied. Applying 0.75V was not efficient in terms of carbon dioxide conversion but under 1.50V 3.0 mole of methane were produced per mole of carbon dioxide the highest ratio obtained for these experiments.

Applying a potential of 1.5V increased the conversion rate of organic matter into methane as well as the ratio between methane and carbon dioxide leading to better biogas quality. The active biofilm formed during the first step of acclimation with activated sewage sludge effectively digested the wastes recovered from an ethanolic fermentation. The digestion was realized in an oxygen free environment in order to force the microorganisms to use the anodes as final electron acceptor to breath. The electrons were driven by the applied voltage to the cathode where hydrogen was intented to be produced by protons reduction. The carbon dioxide could then be reduced into methane on the cathode in the presence of hydrogen[17] increasing the methane content of the biogas produced.

4.5.2 Fermentation waste digestion with in situ H_2 production

A nickel cathode was covered by platinized carbon cloth, filled with phosphate buffer 0.1 M pH 7.0 and separated from the bulk by a Nafion® proton exchange membrane. A potential of 1.50 V was applied between 10 anodes and the central cathode in order to have an in-situ hydrogen production.

The first step consisted of an acclimation of the reactor with the aim to form a biofilm containing methanogens on the anodes. The reactor was acclimated as described in section 3.13.3, the gas composition of the headspace was monitored, the results are presented in figure 64 in the annex 8.5.

At the end of the acclimation phase activated sludge was replaced by wastes recovered from an ethanolic fermentation realized with *S. cerevisiae*. The fermentation was realized in three shake flasks of 1.2 litters (see section 3.13.1), the ethanol production yield was 72.4 ± 8.30%. 23.0 g of fermentation wastes were suspended in 14.0 l of distilled water and added in the reactor.

The gas in the headspace of the reactor was sampled and analysed according to section 3.14.3, the results are presented in the following figure.

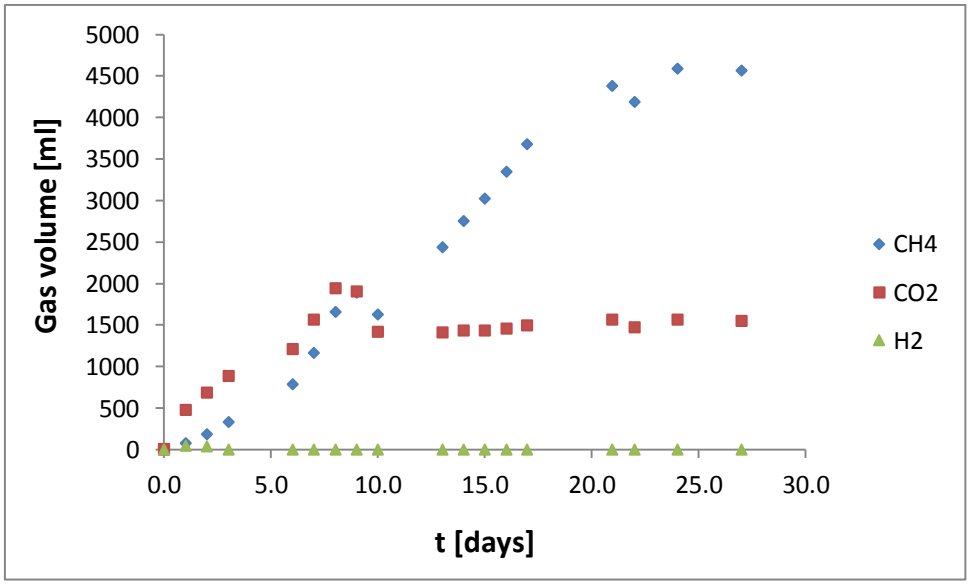

Figure 38 Gas composition in the headspace of the reactor over the in situ hydrogen production experiment.

A small volume of hydrogen was detected in the headspace of the reactor in the early phase of the experiment. At day two 45 ml of hydrogen was present, at day three 34.5ml, from day four to the end of the experiment no hydrogen was detected. No hydrogen was detected with the experiments realised with 9 cathodes made of nickel immersed in the bulk without proton exchange membrane (see section 4.5.1). It is assumed that the presence of hydrogen should lead to the conversion of carbon dioxyde into methane. In fact, in this experiment the ratio between the numbers of mole of methane per mole of carbon dioxyde reached 2.9, one of the highest ratio obtained in the frame of the methanisation experiments. 87.7% of the COD was removed over 26 days, a value close to the one observed with 9 cathodes made of nickel immersed in the bulk (see section 4.5.1). The fraction of COD converted into CH_4 is smaller than the ones observed in previous electromethanisation experiments, 50.6% against 61.3% at 0V, 62.7% at 0.75V and 91.1% at 1.50V with 9 cathodes. By removing 8 cathodes the area available to convert carbon dioxide into methane was reduced and the bulk volume in which no electrodes were present was increased. These differences could favour the conversion of COD into digestable biomass or secondary metabolites, respectivley. The details of the results are presented in table 11.

Table 10: Gas volume and chemical oxygen demand analysis for the bioelectromethanisation experiments realised with 9 cathodes made of nickel and 10 anodes made of RVC.

Number of anodes	Number of cathodes	Applied voltage [V]	V CH_4 [l]	V CO_2 [l]	COD initial [mg O_2/l]	COD final [mg O_2/l]	COD removed [%]	COD converted into CH_4 [%]	Ratio biogas [mole CH_4/mole CO_2]
10	9	0	5.57	1.99	2589	331	87.2	61.3	2.8
10	9	0.75	5.81	2.33	2596	297	88.5	62.7	2.5
10	9	1.50	5.84	1.93	1870	278	89.3	91.1	3.0

Table 11: Gas volume and chemical oxygen demand analysis for the experiments realised with one cathode made of nickel covered with platinized carbon cloth and separated from the bulk by proton exchange membrane. The cathode was filled with phosphate buffer.

Number of anodes	Number of cathodes	Applied voltage [V]	V CH_4 [l]	V CO_2 [l]	COD initial [mg O_2/l]	COD finial [mg O_2/l]	COD removed [%]	COD converted into CH_4 [%]	Ratio biogas [mole CH_4/mole CO_2]
10	1	1.5	4.59	1.56	2109	259	87.7	50.6	2.9

5. Conclusions

5.1 *S. cerevisiae* in MFCs and MECs

It was demonstrated that an electrical current can be recovered from an *S. cerevisiae* fermentation with a bioelectric fuel cell. The yeast fuel cell an electromotive force of 0.43 V was measured (see section 4.1.1). This corresponds to the expected microbial fuel cell power and shows the potential to produce an electrical current. By applying a series of external resistance to the bioelectric fuel cell as a load power densities curves were obtained with a mean maximum power of 176 mW/m^3 (see section 4.1.3). It was demonstrated that the electrical current originated from *S.cerevisiae* metabolism by measuring background currents generated from pure abiotic media (see figure 15).

The electrical currents were recorded in the presence and absence of an exogenous mediator for five different glucose concentrations (170 g/l to 20 g/l). When a mediator was added to the fermentation a higher current dentistry was observed (see figure 20). For glucose concentrations from 20 g/l to 70 g/l the ethanol yields were smaller when a mediator was present. In contrast with 70 g/l to 170 g/l no difference was observed. A series of fermentation were realised in shake flasks without any electrical current production in order to evaluate the impact of the MFC use on ethanol productivity. For each glucose concentration tested the ethanol yields were smaller when an electrical current was produced (see figure 21).

Regarding the hydrogen and bioethanol coproduction a panel of different set-up were tested.

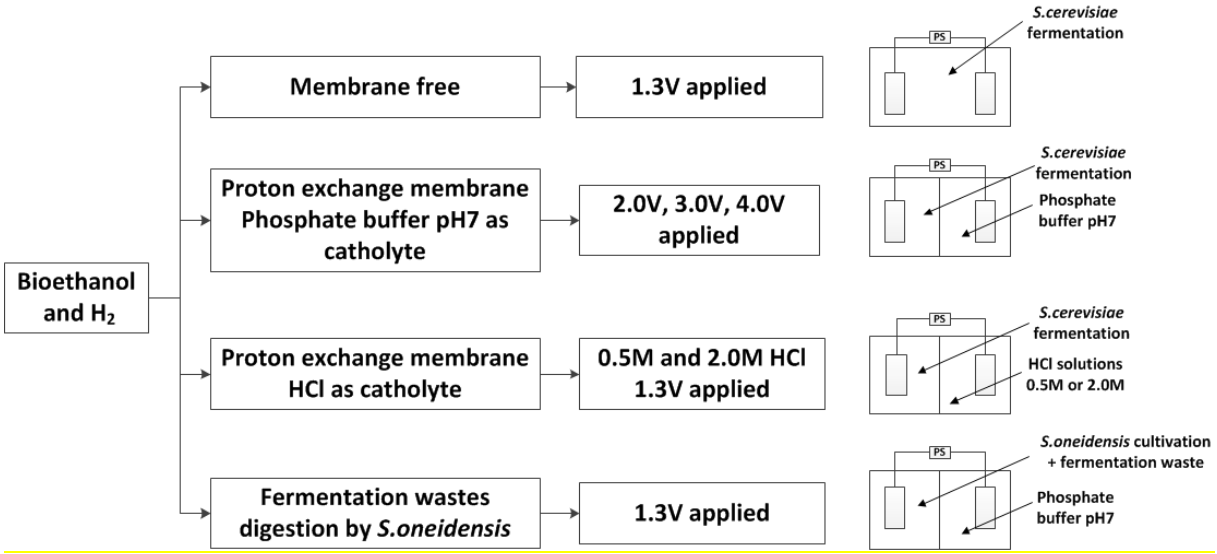

Figure 39: Diagram of experiments realised for hydrogen and bioethanol co-production.

The first set-up tested consisted of the bioelectric fuel cell without membrane with an applied voltage of 1.3V. If hydrogen was produced it could not be detected due to high carbon dioxide evolution from the fermentation (see figure 23). This set-up led to a high ethanol yield of 91.5%.

The second set-up included the use of a proton exchange membrane. The cathode compartment was filled with phosphate buffer 0.1M pH 7. Three applied voltages were tested: 2.0 V, 3.0 V and 4.0 V. Traces of hydrogen were detected under 3.0V and 4.0V (see table 7). Ethanol corresponding production yields were 57%, 58% and 54%. An applied voltage to the system had a negative impact on the ethanol yields and the yeast cells (see figure 29).

Third set-up consisted in the operation of the bioelectric fuel cell in dual chamber mode with HCl (2.0M and 0.5M) used as catholyte with 1.3V applied. This set-up led to the higher hydrogen production observed, 0.48 ml with 0.5M HCl catholyte and 0.67 ml with 2.0M HCl catholyte (see figure 30 and 31). However using such acidic catholytes led to a decrease of the pH in the anodic compartment and a decrease of the ethanol yields. The ethanol yields were 32% with 0.5M HCl as catholyte and 10% with 2.0M HCl catholyte (see figure 32 and 33).

The last set-up tested used the exoelectrogenic properties of *Shewanella oneidensis*. The waste of a 3 litter fermentation (81.9% ethanol production yield) was digested in a acclimated dual chamber microbial electrolysis cell. Once the fermentation waste (Lees and other compounds) was added to the microbial electrolysis cell an increasing current density of about 0.6 mA was observed (see figure 35) and hydrogen was detected in the reactor headspace (see table 9).

This last experiments showed the perspective of fermentation waste digestion. Hydrogen and electrical current are no longer produced directly from the fermentation avoiding negative impact on ethanol yields but from the wastes recovered at the end of the process. At the end of fermentation the biomass recovered contains amino acids, peptides, nucleotides and carbohydrates. Those components could be used as substrate in a microbial electrolysis cell.

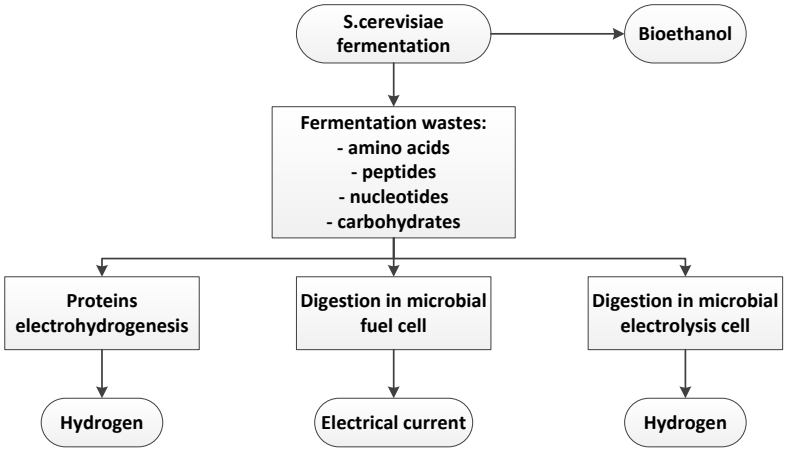

Figure 40: Valorisation of fermentation waste with bioelectric fuel cell system.

5.2 Methanisation of fermentation waste

As described in the previous section waste recovered from a fermentation could be used as substrate. Bioelectric systems can then be integrated in a bioethanol production plant to treat the wastes produced during the fermentation. The ethanol production process and the energy production are then two distinctive steps.

For this last series of experiments, fermentations were realised in shake flasks to produce ethanol. The solid phase of the fermentation was then recovered by centrifugation, lyophilised and used as substrate in an bioelectromethanogenesis cell (see section 3.13.1). In parallel a reactor containing 10 anodes made of RVC and 9 cathodes made of nickel was acclimated. The acclimation was realised with 11.5 l of activated sewage sludge withdrawn from the first digester of a wastewater treatment plant. The aim was to form an active biofilm on the electrodes. The activated sludge was then removed and replaced by wastes recovered from the fermentations realised in shake flasks. The wastes were then digested and converted into methane. The bioelectromethanogenesis cell enhance the biogas quality[17] by converting the carbon dioxide into methane on the cathode in presence of hydrogen and methanogens.

The first fermentation provided an ethanol yield of 64.2±11.1 %, 23 g of wastes recovered from this fermentation were then digested in the reactor without applied voltage. 5.57 l of methane were produced over 36 days, 87.2% of the COD initially present was removed, 61.3% of the COD consumed was converted into methane, and the ratio between the numbers of mole of methane per mole of carbon dioxide was 2.8

The second fermentation reached an ethanol production yield of 71.9 ± 3.80%, 23 g of wastes recovered from this fermentation were suspended in 11.5 l of distilled water and digested under 0.75 V. 5.8 l of methane were produced over 36 days, 88.5% of the COD contained in the wastes was consumed, 62.7% of the COD consumed was converted into methane and 2.5 moles of methane were produced per mole of carbon dioxide.

The third fermentation had an ethanol production yield of 63.5 ± 11.1 %, 23 g of wastes recovered were added in the reactor. 5.84 l of methane were produced, 89.3% of the COD was removed over the 36 days of digestion and 91.1 % of the COD removed was converted into methane for a ratio mole of methane per mole of carbon dioxide of 3.0.

In this series of experiments realised with 10 anodes made of RVC and 9 cathodes made of nickel it was demonstrated that applying a voltage between the electrodes can increase the biogas quality. Under 1.50 V 91.1 % of the COD consumed was converted into methane against 62.7 % under 0.75 V and 61.3% when no voltage was applied. The highest ratio mole CH_4/mole CO_2 was achieved by applying 1.50 V, the ratios were respectively 2.5 and 2.8 under 0.75 V and 0.0 V. The increase of the voltage seems to be

essential for the conversion of carbon dioxide into methane on the cathodes. Higher voltage leads to hydrogen production[17], this hydrogen can combine with carbon dioxide to produce methane.

A last experiment was realised with a single cathode covered with platinized carbon cloth. The cathode was separated from the bulk by proton exchange membranes, a phosphate buffer pH 7.0 was used as catholyte.

The fermentation realised for this experiment had an ethanol content of 72.4 ± 8.30%. 4.59 l of methane were produced over 27 days, 87.7 % of the COD initially present was consumed, 50.6 % of the COD consumed was converted into methane and the ratio mole CH_4/mole CO_2 was 2.9. For this last experiment a smaller volume of biogas was produced, in fact 6.5 l of biogas were generated against 7.6 l when no voltage was applied, 8.4 l under 0.75 V and 7.8 l under 1.50 V (with 9 cathodes and 10 anodes). By removing 8 cathodes from the bulk the area available to catalyse the conversion of carbon dioxide into methane was reduced. This reduction had a negative impact on the volume of biogas produced but not on its quality. In fact, the ratio between the number of mole of methane produced and the number of mole of carbon dioxide reached 2.9, one of the highest for these methanisation experiments.

6. Perspectives

The three energy vector production in a bioethanol microbial fuel cell type mini-system shows that simultaneous electricity and ethanol production is possible. The electricity obtained is a minor contributor but an option. In contrast hydrogen generation directly from yeast cultivation is not an option as the applied power needed is in no relation to the energy gain in form of hydrogen gas. Therefore the focus should be directed to the valorisation of the wastes produced during the fermentation process. The waste can be used as substrate in a microbial fuel or electrolysis cell using an optimised biocatalyst like *S. oneidensis* (see section 4.4). As seen in this work bioelectromethanogenesis could be an option since good quantities of methane can be produced from such wastes rich in organic matter (see section 4.5). The in-situ hydrogen production experiment realised with a single cathode covered with platinized carbon cloth resulted in a biogas quality comparable to the experiments realised with 9 cathodes made of nickel (see section 4.5.2). High conversion rate of the organic matter present in fermentation wastes into methane could be achieved through cathode composition and set up optimization.

7. Reference

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8. Annex

8.1 Bioelectric fuel cell characterization in microbial fuel cell mode

	P_{max} [mW/m^3] Volume	P_{max} [mW/m^2] True surface area	P_{max} [mW/m^2] Opposite side area	R at P_{max} [Ω]	R int. [Ω]
Run 8	216.26	0.0268	2.514	761.25	941.64
Run 10	160.67	0.0291	2.731	790.53	857.38
Run 11	150.49	0.0254	2.388	1146.5	1063.9
Average	175.81	0.0271	2.544	899.43	954.31
STD dev.	28.91	0.0015	0.142	175.12	84.79
Confidence interval (95%)	71.80	0.0038	0.352	435.01	210.62

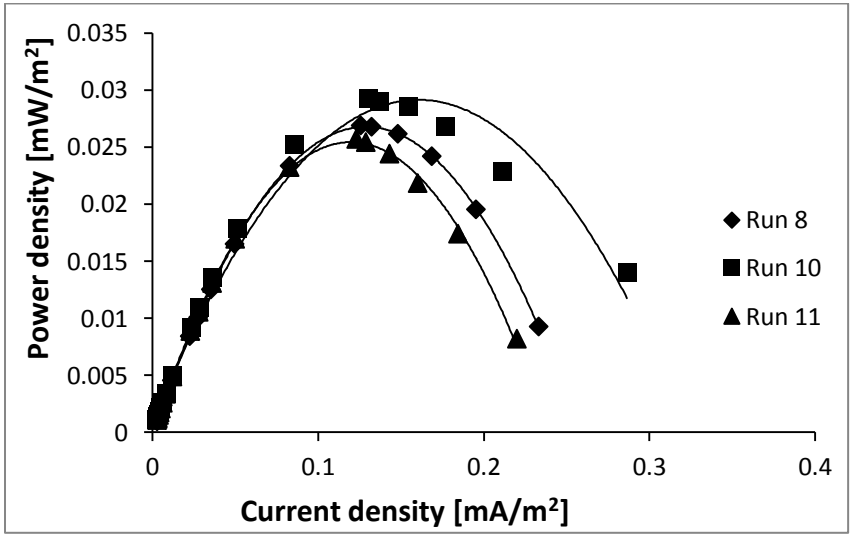

Figure 41: Power density curves normalized to the true surface area of the anode.

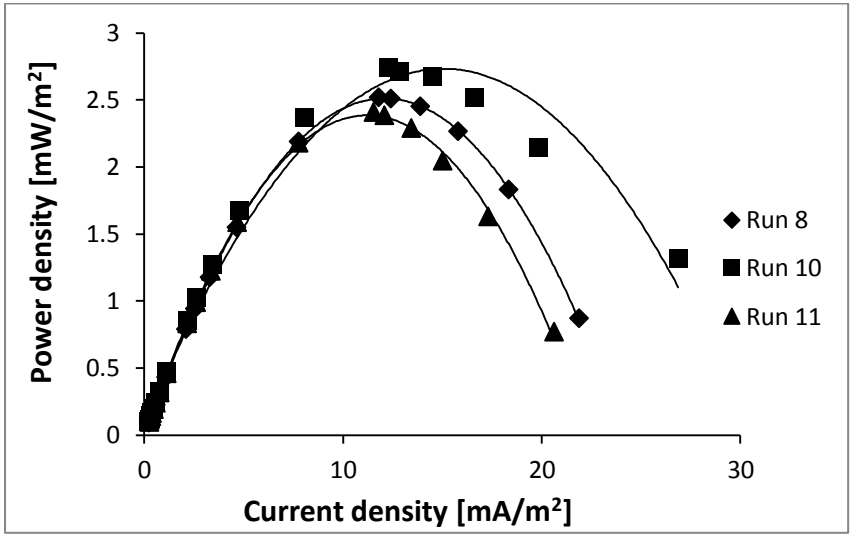

Figure 42: Power density curves normalized to the anode opposite surface area.

8.2 Ethanol productivity in blank experiments realised in shake flasks

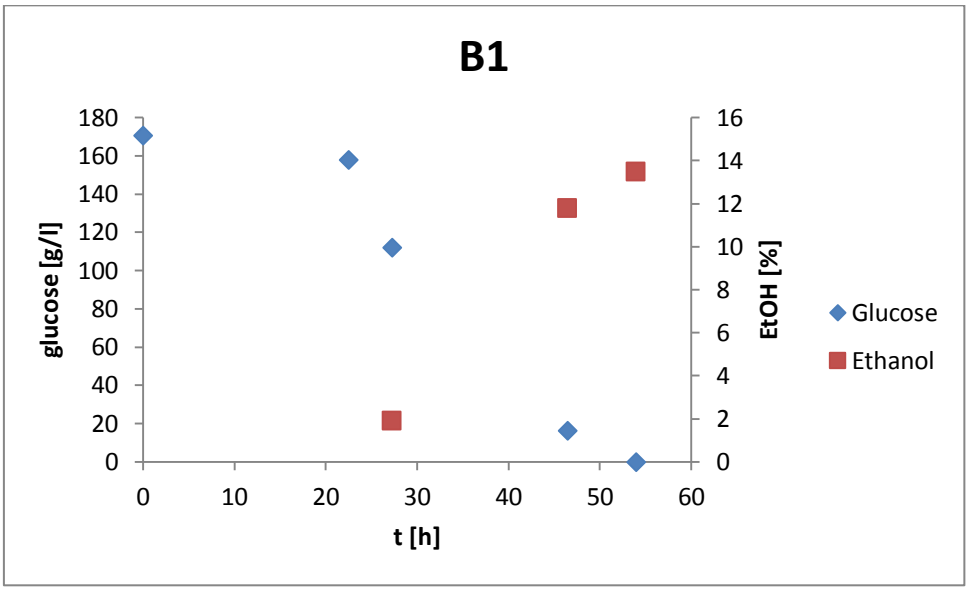

Figure 43: Evolution of the glucose and ethanol concentrations for the fermentation realised in shake flask with an initial glucose concentration of 170 g/l.

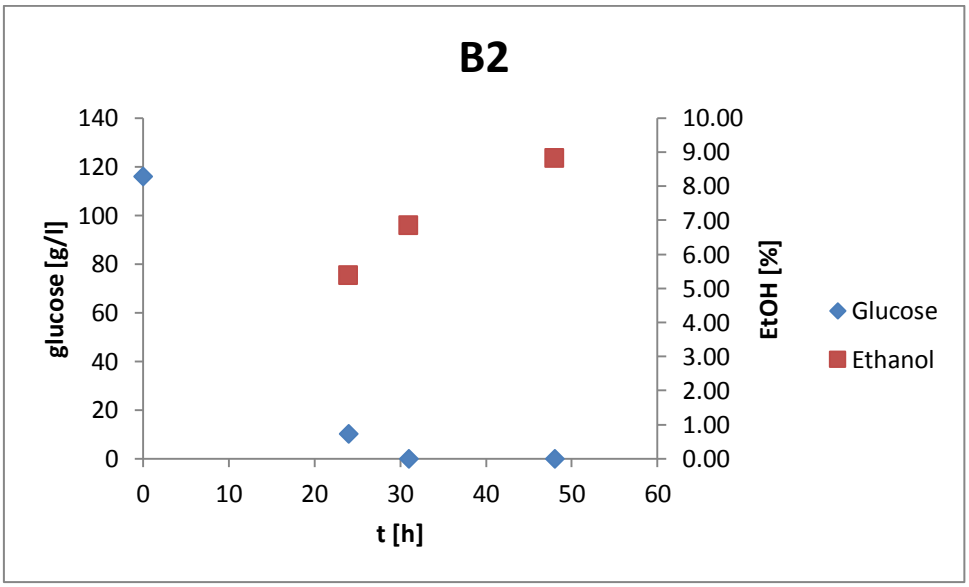

Figure 44: Evolution of the glucose and ethanol concentrations for the fermentation realised in shake flask with an initial glucose concentration of 120 g/l.

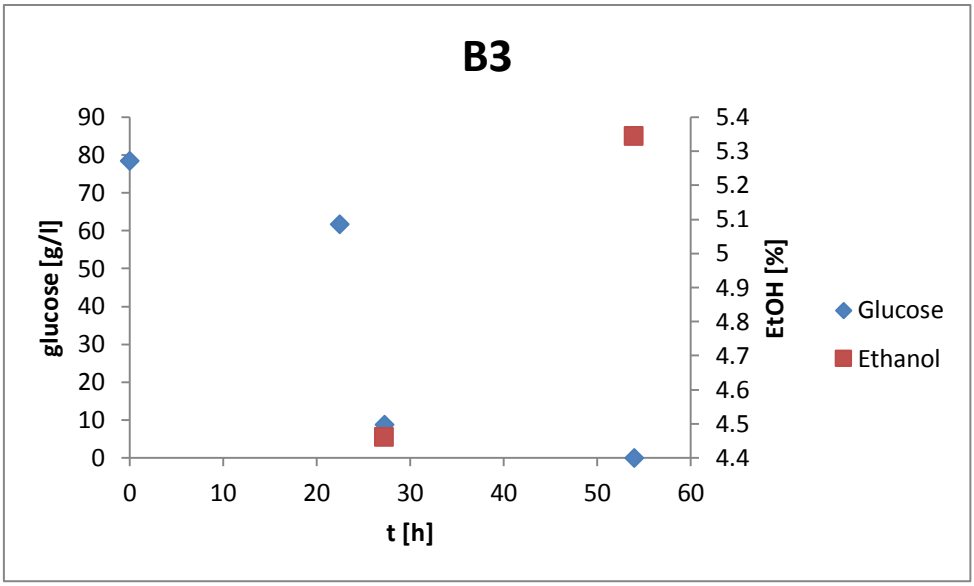

Figure 45: Evolution of the glucose and ethanol concentrations for the fermentation realised in shake flask with an initial glucose concentration of 70 g/l.

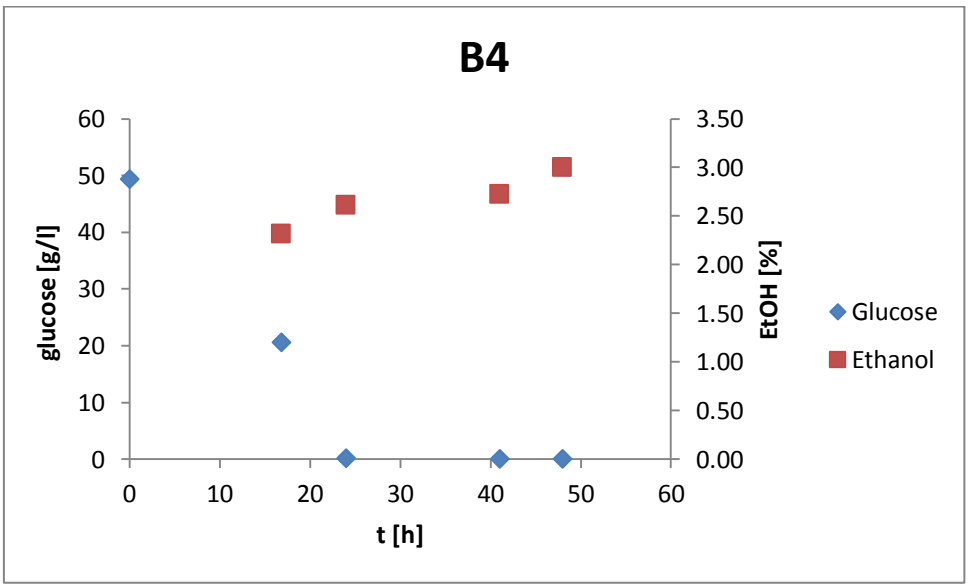

Figure 46: Evolution of the glucose and ethanol concentrations for the fermentation realised in shake flask with an initial glucose concentration of 50 g/l.

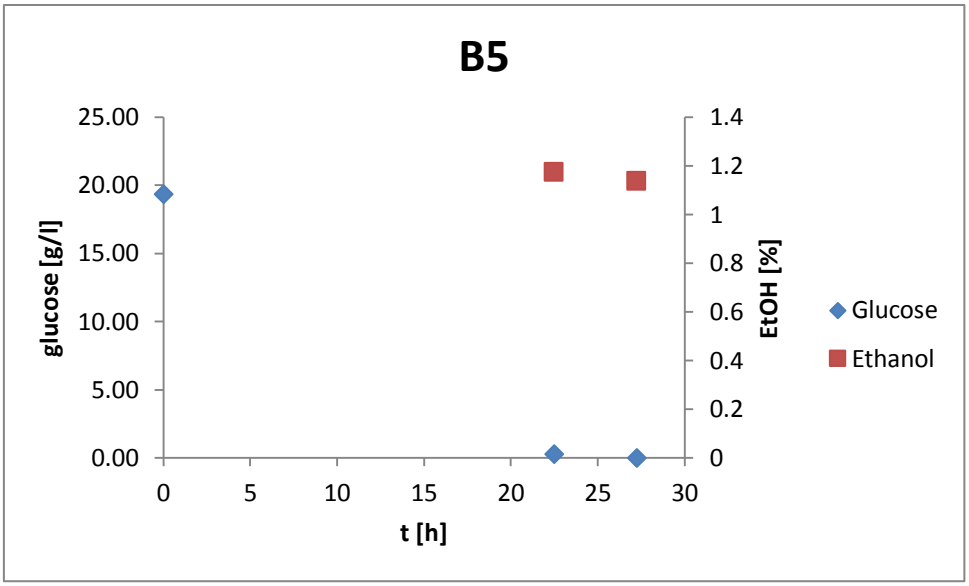

Figure 47: Evolution of the glucose and ethanol concentrations for the fermentation realised in shake flask with an initial glucose concentration of 20 g/l.

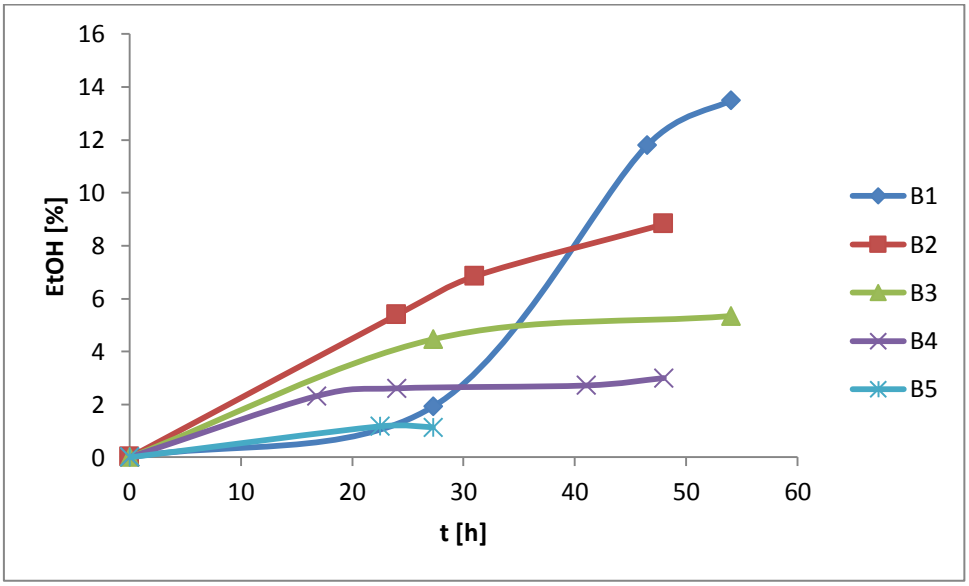

Figure 48: Compilation of the ethanol concentrations for the blank experiments realised in shake flasks.

8.3 Current and ethanol co-production with *S. cerevisiae* in absence of mediator.

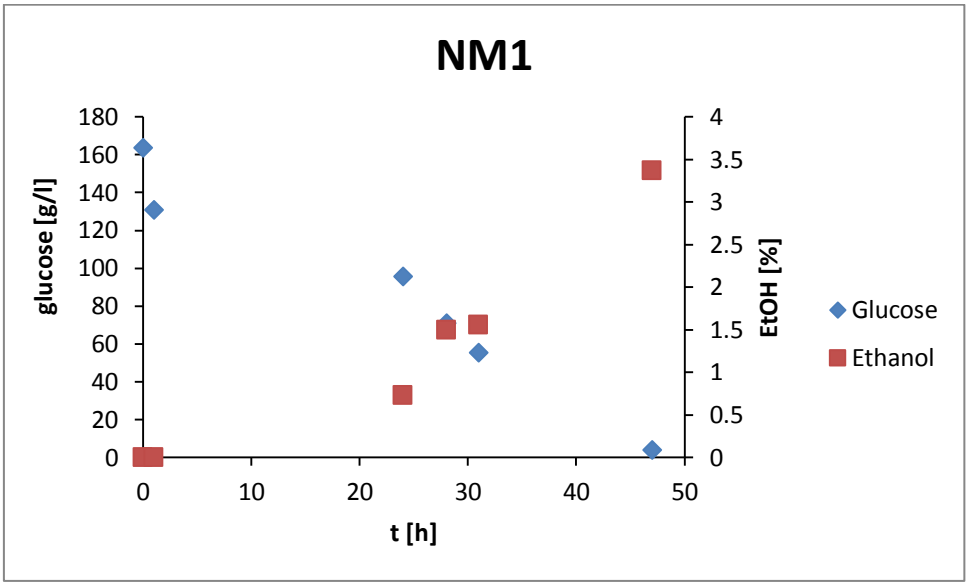

Figure 49: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode without mediator with an initial glucose concentration of 170 g/l.

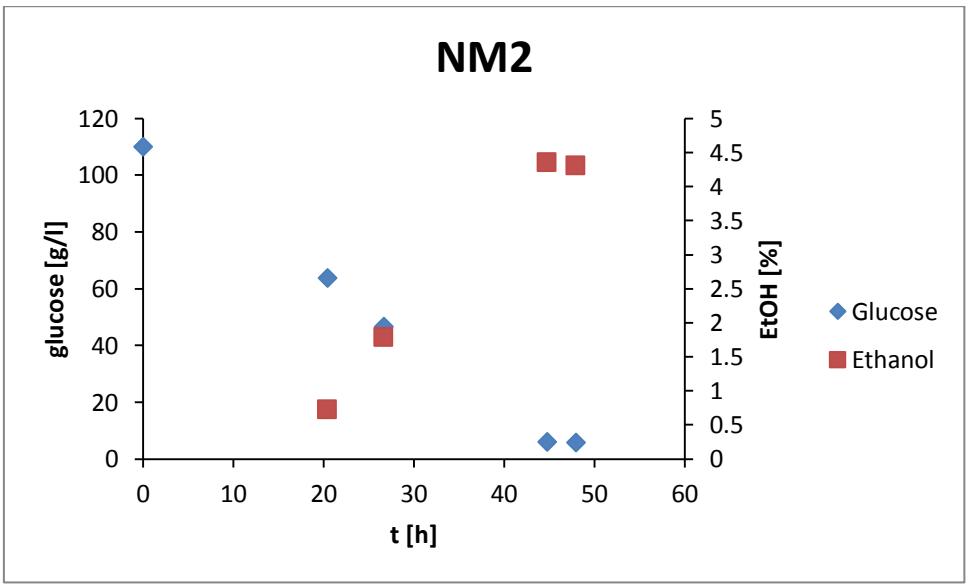

Figure 50: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode without mediator with an initial glucose concentration of 120 g/l.

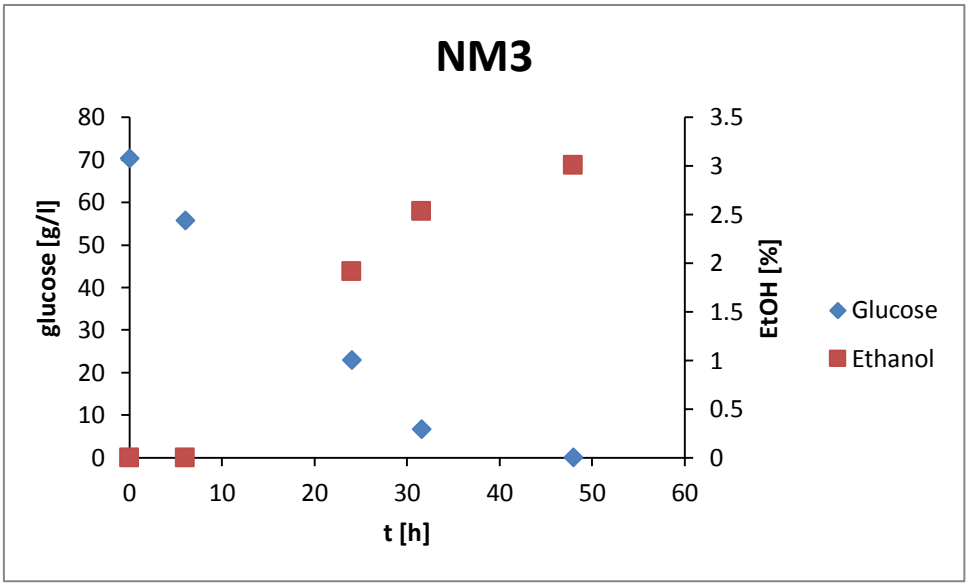

Figure 51: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode without mediator with an initial glucose concentration of 70 g/l.

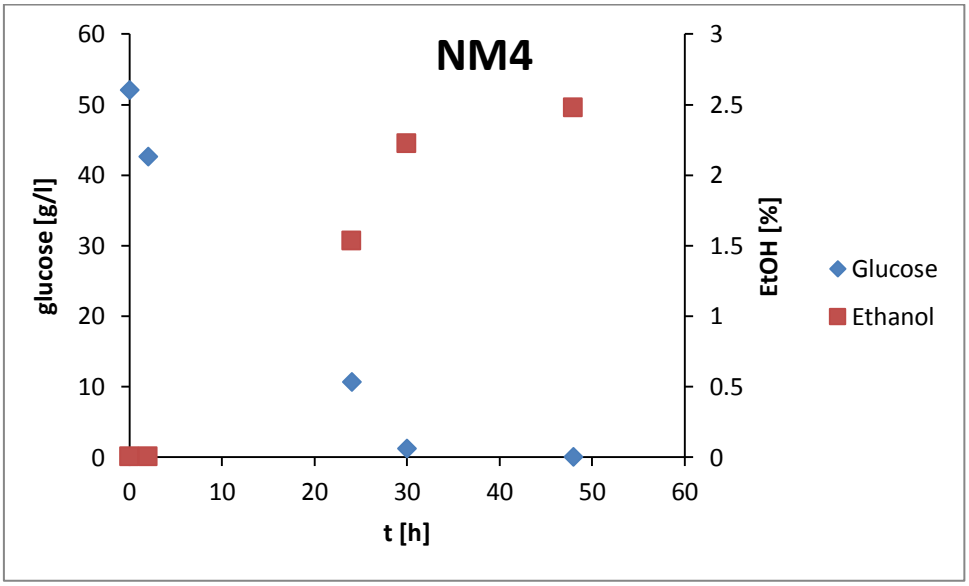

Figure 52: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode without mediator with an initial glucose concentration of 50 g/l.

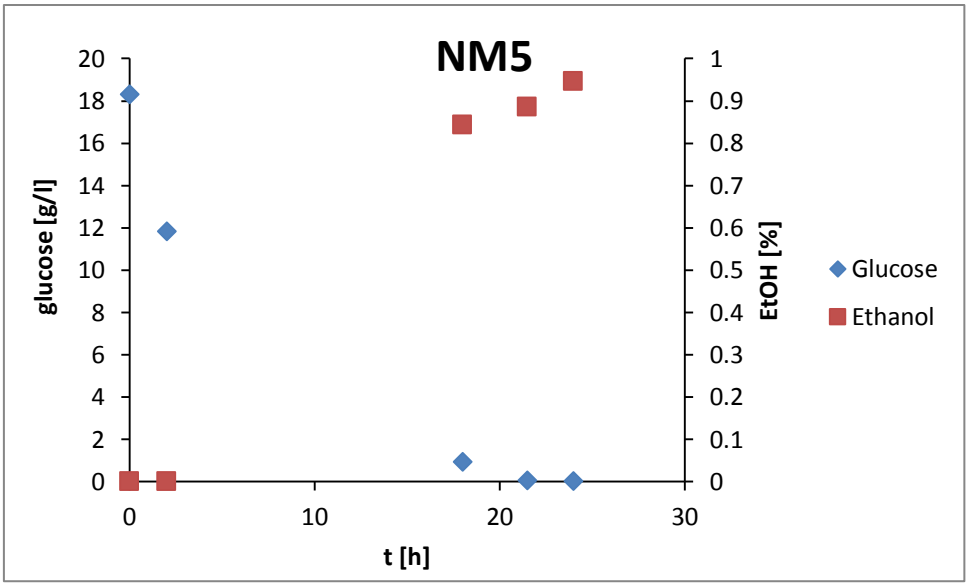

Figure 53: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode without mediator with an initial glucose concentration of 20 g/l.

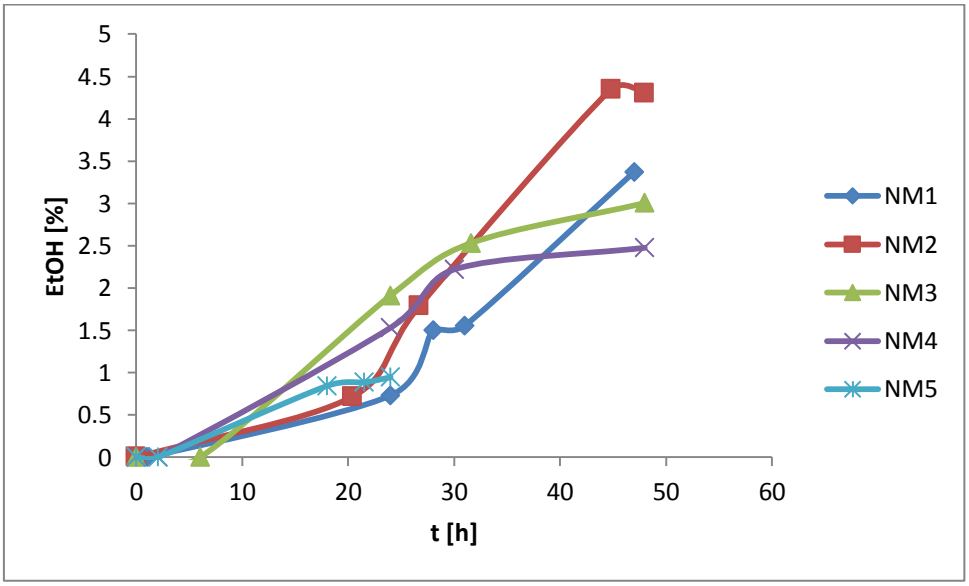

Figure 54: Compilation of the ethanol productions for the experiments realised without mediator in microbial fuel cell mode.

8.4 Current and ethanol co-production with *S. cerevisiae* in presence of mediator.

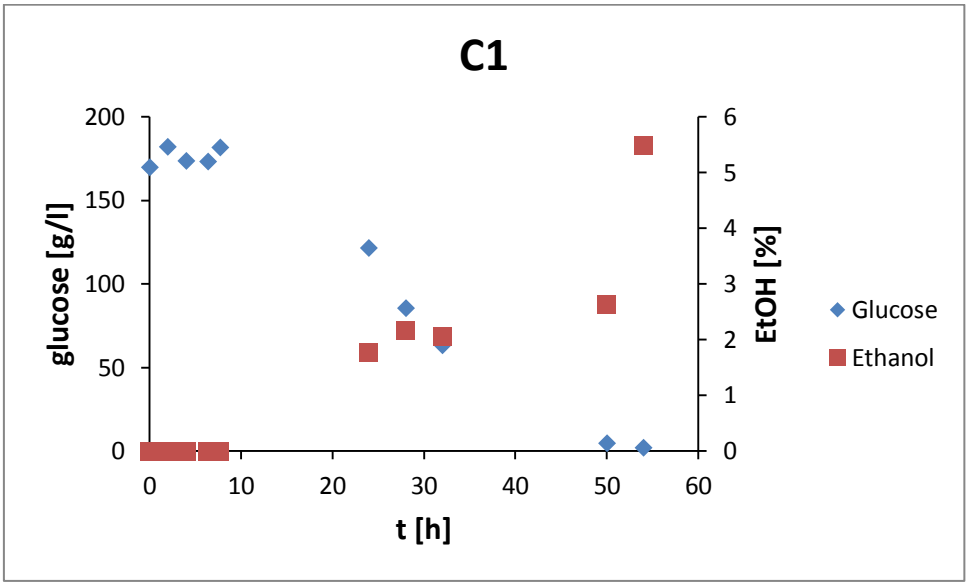

Figure 55: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode with mediator and an initial glucose concentration of 170 g/l.

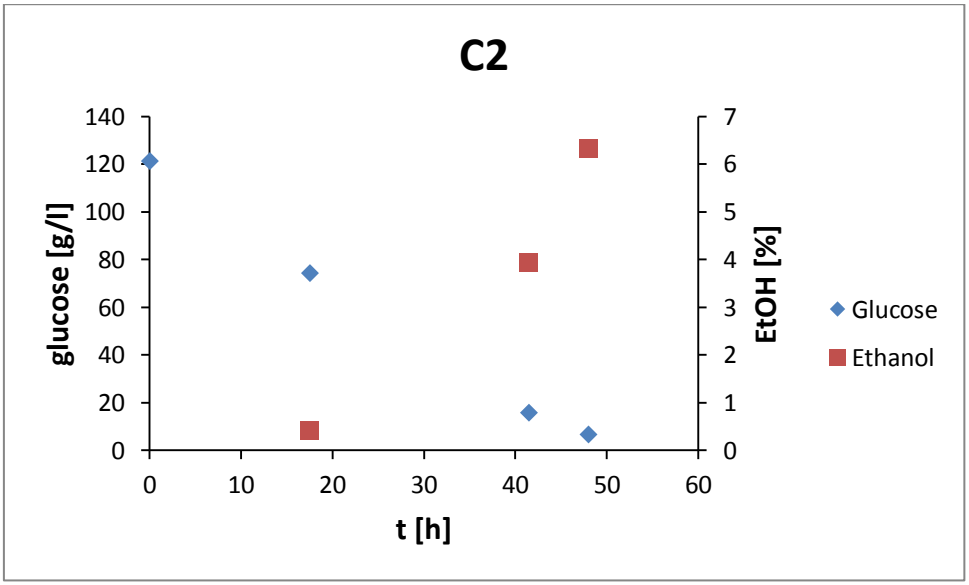

Figure 56: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode with mediator and an initial glucose concentration of 120 g/l.

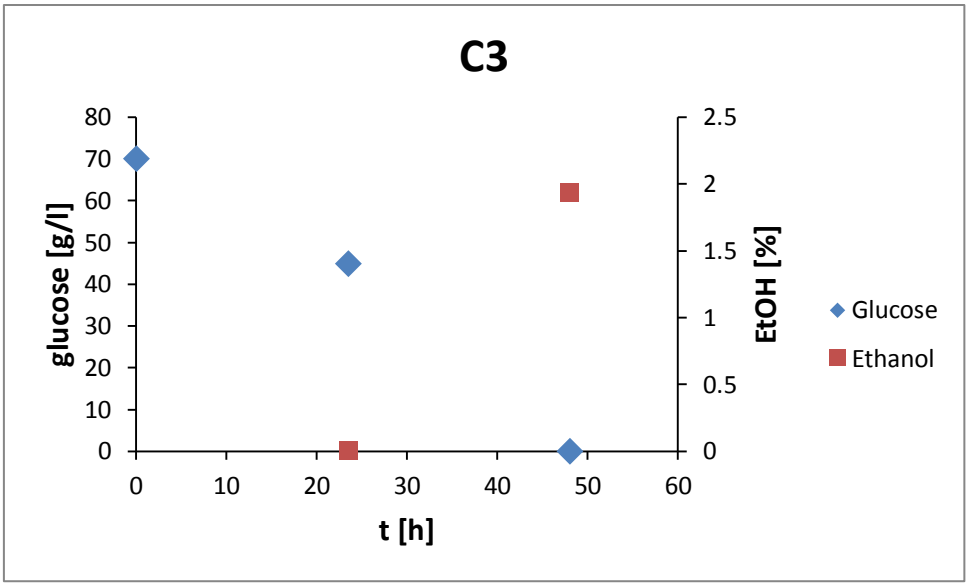

Figure 57: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode with mediator and an initial glucose concentration of 70 g/l.

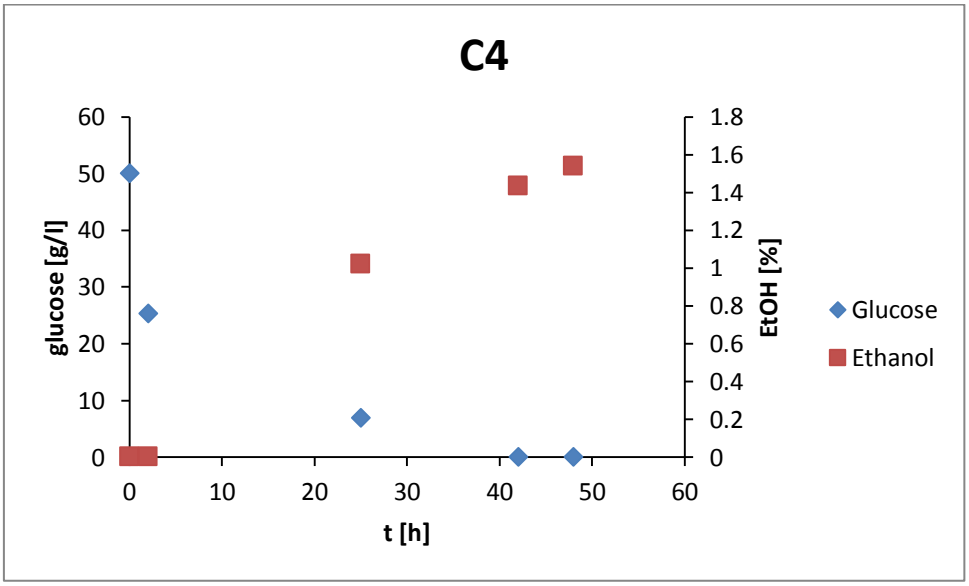

Figure 58: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode with mediator and an initial glucose concentration of 50 g/l.

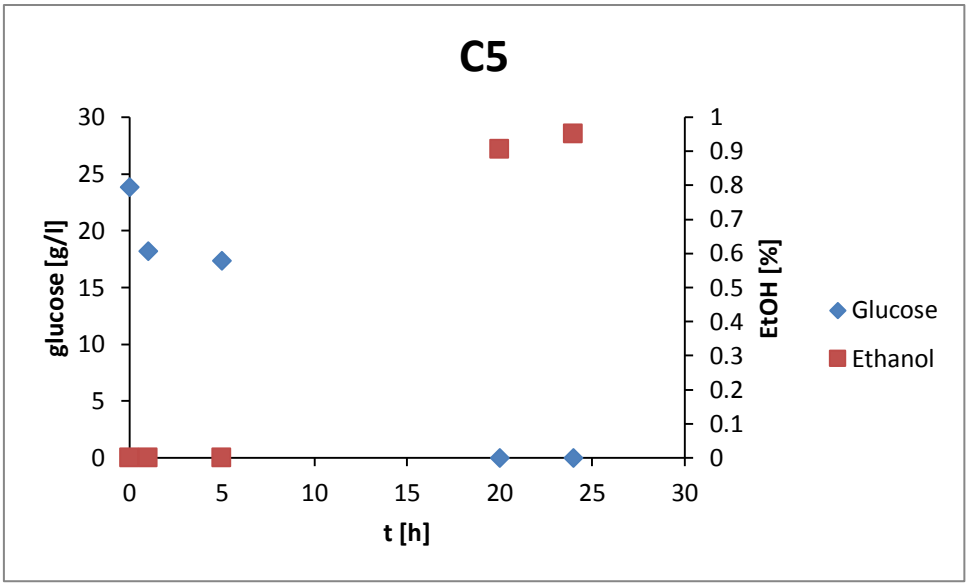

Figure 59: Evolution of the glucose and ethanol concentrations for the fermentation realised in microbial fuel cell mode with mediator and an initial glucose concentration of 20 g/l.

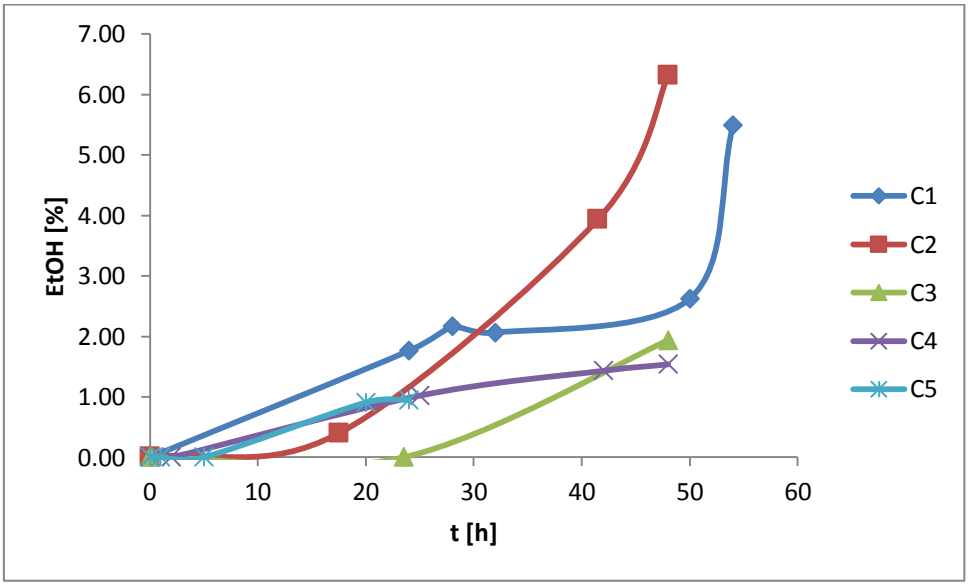

Figure 60: Compilation of the ethanol productions for the experiments realised with 0.3 mM Methylene Blue as mediator in microbial fuel cell mode.

8.5 Acclimation of reactor for methanisation

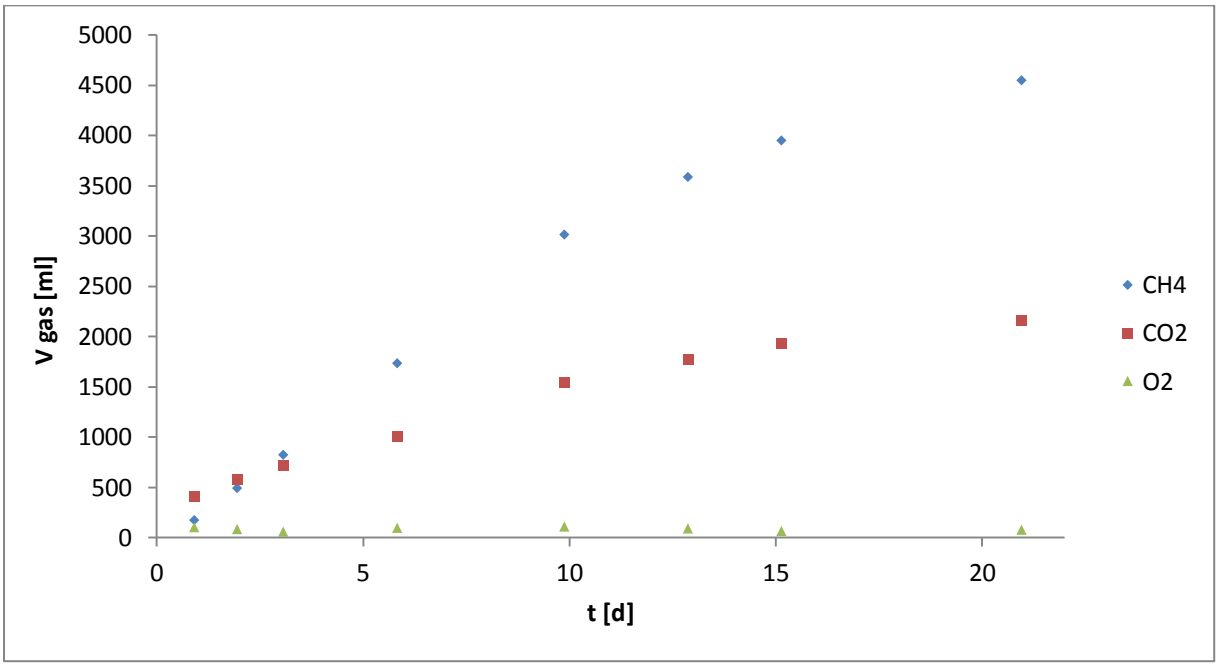

Figure 61: Gas production over the acclimation phase preceding the digestion of fermentation waste under 0V.

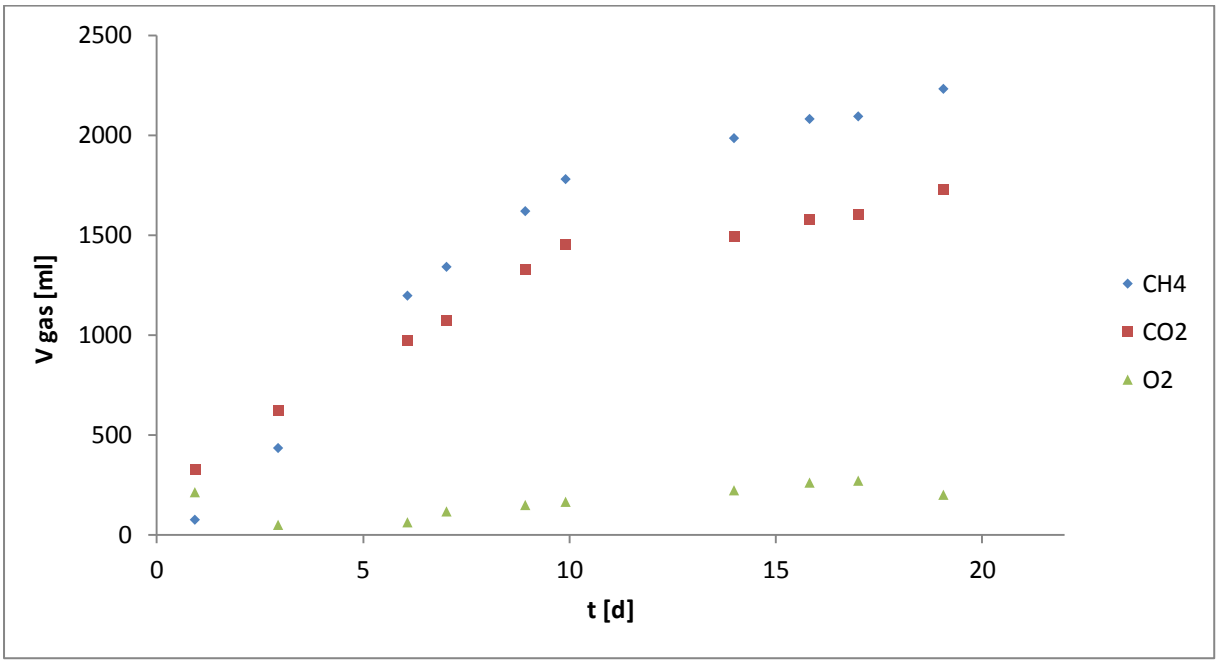

Figure 62: Gas production over the acclimation phase preceding the digestion of fermentation waste under 0.75V.

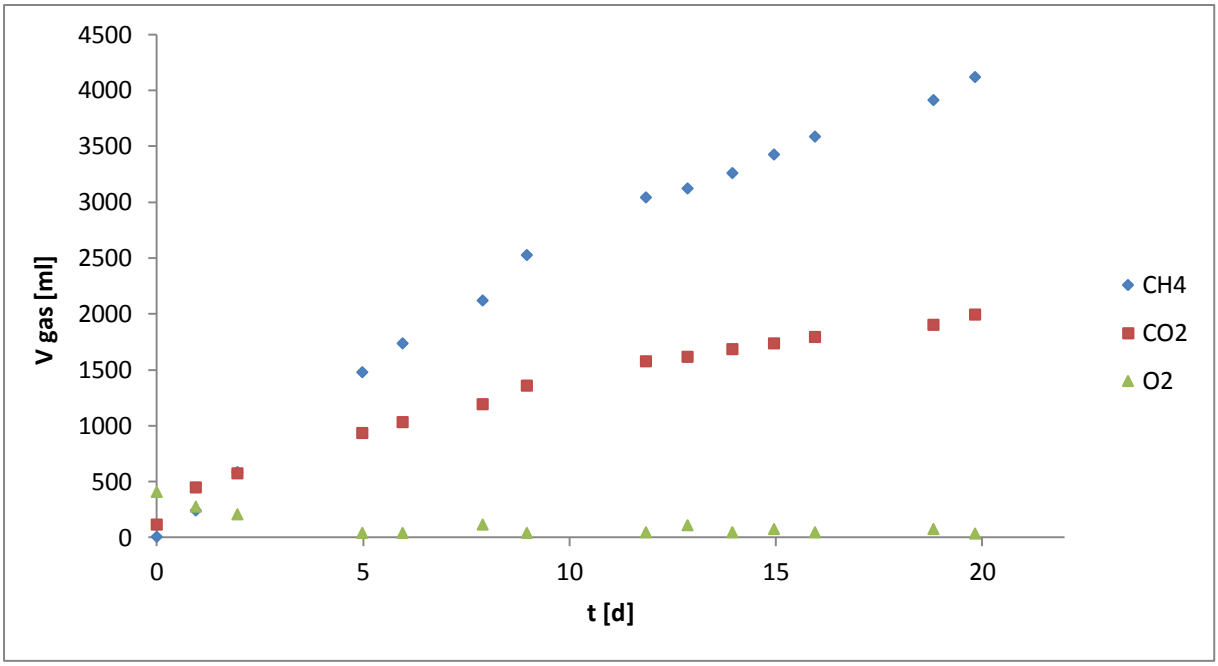

Figure 63: Gas production over the acclimation phase preceding the digestion of fermentation waste under 1.5V.

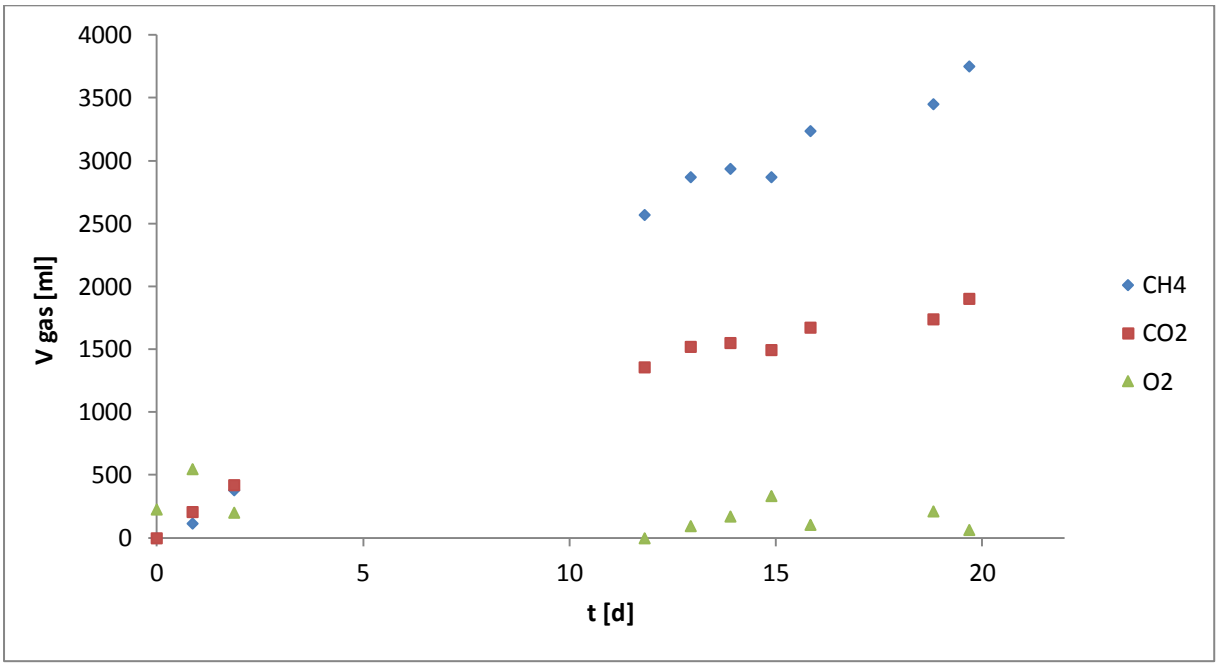

Figure 64: Gas production over the acclimation phase preceding the digestion of fermentation waste with in situ hydrogen production.