

Electrogenic characterization and optimization of the cyanobacterium *Anabaena sp.* and construction of a Photovoltaic Microbial Fuel Cell

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Abstract. Recent advances in electrogenic capabilities of autotrophic microorganisms have given an optimistic alternative for future renewable energy sources[1]. Cyanobacteria *Anabaena sp.* is currently used as a fertilizer due to its nitrogen fixing ability. However, its application as an electricity generator is not exploited because of the low throughput that is obtained.

A better understanding of the relation cells-electrode was intended in this project and optimization of the current signal provided by the bacteria was also studied. The presence of an electro-active natural mediator has been demonstrated and stress induction has appeared as an interesting factor to increase the obtained current. Finally, in order to study the application of *Anabaena sp.* in the anode of a fuel cell, a Photovoltaic Microbial Fuel Cell was constructed.

INTRODUCTION

Global energy demand is increasing every year even more than population does. The amount of electricity consumed in our planet nowadays is $1,31 \times 10^{14}$ kW per hour ($5,7 \times 10^3$ kW per hour is the demand per person in Spain) and fossil fuels have been the main source to satisfy this requirement so far [2].

Even though they are efficient, these fuels come with two important drawbacks: (i) petroleum, coal and gas, which are the three components of fossil fuels, are getting depleted and (ii) their combustion generates large quantities of carbon dioxide which is the main cause of the green-house effect. Consequently, different CO₂-neutral and renewable energy sources have appeared in the last decades as profitable alternatives to fossil fuels[3]. This work focuses in those alternatives converting sunlight to electricity, called photovoltaic devices (sunlight provides to the earth $2,03 \times 10^{10}$ kW/hour) [4]. Especially it considers those devices involving autotrophic bacteria.

Microorganisms have an extended capability to exchange electrons with the environment, either donating or receiving them [5][6][7]. The electric charges travel continuously from some molecules to others. It happens in photosynthesis, where the electrons from the

water splitting are transferred to cytochromes and ferredoxine; and energy is obtained by the cells. When an excess of excited electrons are present in autotrophic bacteria, these can be redirected to an external acceptor, the electrode[8]. This transfer of electrons from cells to electrodes has potential applications in electricity generation.

Previous studies have given an insight in the process. Light dependency of the electrogenic activity of cyanobacteria was demonstrated in the 80s when the first evidences of this application were investigated [9]. Later on, in order to determine the exact origin point of the electrons in the photosynthetic reaction chain, some enzyme inhibitors were used [10]. The variety of results though, didn't end in a robust conclusion. Furthermore, R. Bradley et. al. genetically modified *Synechocystis sp.*, knocking out two thylakoid-localized terminal oxidases genes and increased the yield obtained by 3-fold [11].

Some research groups attempted to demonstrate how the electrons are transferred from cells to electrodes [12][13]. Natural (hydrogen or quinones) and artificial (viologens or vitamin K3) mediators appeared to serve as good molecules to carry the charges [14][15]. Although conductive pili between cells have been observed in cyanobacteria, direct electron

transfer from cells to electrodes has only been reported in heterotrophic microorganisms[16].

Since most of the previous works were conducted on *Synechocystis* sp. [17][11] we decided to use a different bacterial strain. We used the cyanobacterium *Anabaena* sp. which it has also been described to present electrogenic activity [4]. It is a filamentous cyanobacteria that exists as plankton in freshwater ponds and backwaters; and it is also interesting due to the presence of heterocysts which are specialized cells, located at semi-regular intervals along the filaments, that can fix atmospheric nitrogen into ammonia [18].

Our aim was to provide a better understanding of this cyanobacterium and its electronic features. We wanted to characterize the capacity of *Anabaena* sp. to donate electrons and optimize the signal measured for its electric generation applications.

In order to study this strain, we used an electrochemical cell with a three electrode configuration: working (WE), counter (CE) and reference (RE) (Figure1). It is a well-known setting that provides reliable information about the redox reactions [19]. Basically, the potential of the electrode is switched from negative to positive values and the response is observed as current values.

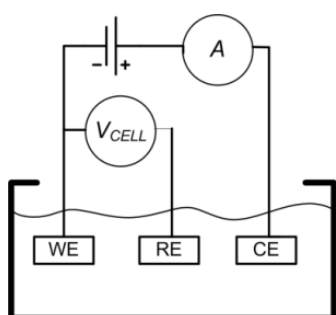


Figure1. Three electrode configuration

After characterization of *Anabaena* sp., our second aim based on the construction of a Photovoltaic Microbial Fuel Cell (PMFC). It was built for future purposes, to study the electric generation capacity of our strain. In a three electrode configuration, we must apply electric potential to study cell-electrode interaction, in a PMFC however, oxidation in one electrode is

coupled with a reduction reaction in a second electrode and net electric energy is obtained.

The fuel cell is composed by two chambers separated with a Proton Exchange Membrane and an electrode in each one, the anode and the cathode. Oxidation and reduction respectively. The PMFC allows the electrons coming from the cyanobacteria to be reduced [20]. It can be seen as a battery that is runned with microorganisms (Figure2).

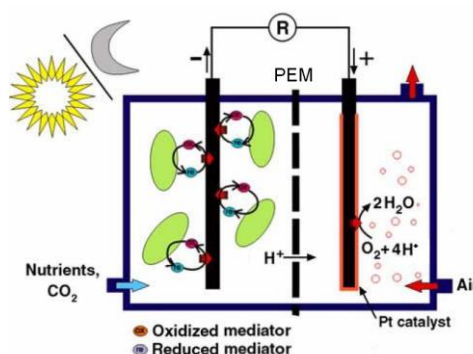


Figure2. Photovoltaic Microbial Fuel Cell Scheme

Even though an inorganic reaction usually serves as the reductive process (Figure2), biocathodes have also been studied. Different microorganisms present the capacity to harvest electrons coming from an electrode and use them for its own metabolism. This combination avoids the necessity to use metallic catalysts in the cathodes (Platinum) [21].

PMFC offers some advantages when compared to similar technologies. In contrast with current solar panels, cyanobacteria are self-sustainable and self-repairing organisms really inexpensive to be grown [22]. When compared to fuel cells using heterotrophic organisms (HMFCs), Photovoltaic MFCs don't need a continuous supply of organic matter as the carbon source. Furthermore, the energy of the electrons obtained in PMFCs is higher than those coming from the by-products of metabolites respiration (HMFCs case). Being the photosynthetic reaction chain the origin of the electrons implies that they are in its highest energetic level, regarding biological means. From photosynthesis on, these same electrons will be powering the formation of organic matter that afterwards, heterotrophic organisms will

consume. Electrons are transferring and so losing energy in every step [23][24].

The main disadvantages that don't allow PMFCs to be implemented as electric factories are the low power outputs and the high costs of the materials usually used for constructing them.

This work attempts to provide a better understanding of electron transfer processes and PMFCs construction methods in order to solve both these problems.

MATERIALS AND METHODS

Culture and cyanobacteria growth

The experiments were conducted on *Anabaena* sp. BEA0858B. The strain was maintained at 21 +/- 2 °C under stirring conditions. It was kept in sterile containers with filters located in the caps that allowed oxygenation; under cycles of 16h Light; 8h Dark (4x15W/m² fluorescent lights). The culture was grown in recommended media BG-11 and it was checked twice a week for contamination using two different methods: visual observation under microscope (Nikon Y-FL HB-10104AF) and agar-plate culturing with yeast extract where heterotrophic organisms would grow if present. Culture growth was monitored using a spectrophotometer (BioNova Científica S.L. Spectra max 340PC) at 680nm of wavelength for chlorophyll determination. This parameter was correlated with the amount of cells per micro-liter using a Neubauer chamber. For analyzing, 20ml of sample were taken, in sterile conditions.

Electrochemical cell and measurements

A 30ml glass container was used as three electrode electrochemical cell. To fit in the three electrodes, three holes were drilled in the cap. All the experiments were carried out using a Ag/AgCl reference electrode (CHI Instruments Ag/AgCl Reference electrode), a platinum mesh (5mm x 10mm) as the counter electrode and a graphite bar with constant active area as the working electrode.

Cyclic voltammetry (CV) was used for electrochemical analysis with different scan rates (from 1 to 100mV/s) and potential ranges (from -0,2 to +0,7V) using a potentiostat (Ecochemie-Autolab PGSTAT10). The chamber was filled with BG-11 medium together with the sample to be analyzed and the medium itself and/or distilled water were used as the negative control.

ChronoAmperometry (CA) was also conducted with the same configuration of electrodes as CV and a fixed potential of +0,6V vs Ag/AgCl. The amount of sample used for testing was 20ml and absorbance and pH were also measured.

The results of CVs and CAs are presented in different formats throughout the report, depending on what the study focuses in.

In CVs there are two parameters which are mainly relevant: the height of the peaks and its position in the X axes. The height is an intensity value and it is proportional to the concentration of molecules that are reacting. It is used as a quantitative value to express electrogenic activity. The position is expressed in Volts and it corresponds to the formal potential of the redox reaction under analysis.

In CAs, bar graphs are used to express the intensity values obtained.

Surface area calculation

Active surface area of the working electrode could be calculated from Randles-Sevcik equation (1). Using CV in 2mM Fe(CN)⁻³₆ / 1M KNO₃ solution and a potential range of +0V to +0,6V. The reduction peak's intensities were plotted against the square root of the scan rates (25, 50 and 100mV/s) and by performing linear regression the slope (*k*) could be obtained. One can express the area as a function of *k* (2).

$$i_p = (2.69 \times 10^5) n^{3/2} D^{1/2} CA v^{1/2} \quad (1)$$

$$A = k / ((2.69 \times 10^5) n^{3/2} D^{1/2} C) \quad (2)$$

n stands for the number of electrons exchanged in the reaction. Since ferricyanide is converted to ferrocyanide, just one electron is participating in the system. *D* is the diffusion

coefficient ($6.70 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), C is the molar concentration of the solution (mM), A is the active surface area (cm^2) and v is the scan rate (V s^{-1}) [25].

Equipment and reagents

Different equipments and instruments were also used for different applications reported: pH-meter (CRISON Basic20), centrifuge (MPW 350R), grinding machine (MiniBeadBeater – Biospec), dialysis membrane (Sigma-Aldrich D9777-100FT), tester instrument (Digital Multimeter DT830D), spectrophotometer (BioNova Cientifica S.L. Spectra max 340PC) and the air compressor (TurboJet M-101 - ICA). Glucose (Glucose 99%, Sigma Aldrich) and Ferrocyanide (PamReac – MontPlet&Esteban SA) were the reagents utilized.

RESULTS

01. Characterization:

Electrodes performance and characterization

Graphite bars of 3mm in diameter and 6cm long were cut to be used as working electrodes. They were introduced in heat shrinking tubes so the whole bars were isolated with exception of the edges. When performing experiments, the electrodes were half introduced into the solution and the part that remained outside was connected to the potentiostat with an iron crocodile.

Low resistances ranging from 1 to $1,5\Omega$ were observed using a tester instrument. The active surface area was obtained to calculate the current density (intensity/active area). We used CV and the Randles-Sevcik equation. It ranged from $0,45$ to $0,55\text{cm}^2$.

Determination of the presence of electro-activity

Cyclic Voltammetry is a versatile technique that allows us to see the presence of any electro-activity in the medium [26]. When either direct electron transfer or mediated electron transfer

occur, it appears in voltammograms as intensity peaks. These peaks excel from the background noise or capacitive current that is always present when a voltage is applied.

We compared the medium BG-11 as the negative control with a cyanobacteria culture. The results show both the oxidation and the reduction peak only when bacteria cells are present (Figure3). It demonstrates electro-activity dependence of the cyanobacteria.

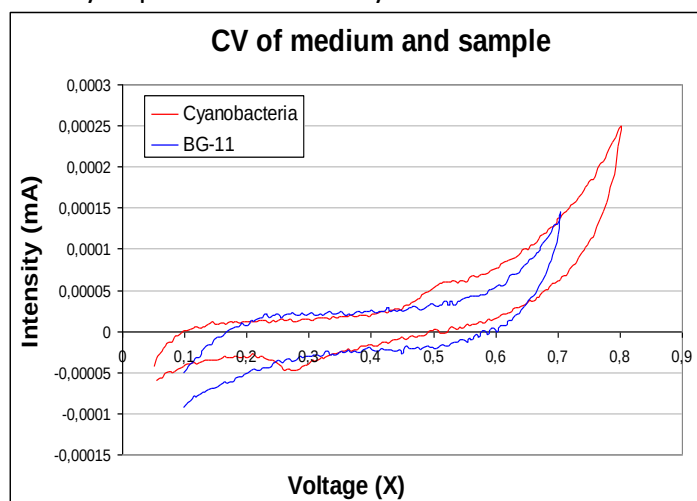


Figure3. Cyclic Voltammogram comparing BG-11 medium as negative control and cyanobacteria. A scan rate of 1mV/s was used.

Quantitative analysis of the voltammogram

Different information such as the reversibility of the reaction, the rate limiting factor (electron transfer or diffusion), or the formal potential can be elucidated from Cyclic Voltammetry [27]. All these parameters give an insight in the electro-active process and its potential application to Fuel Cells.

For this experiment, absorbance of the sample was measured using a spectrophotometer. It was 1,236 (680nm) which could be correlated with the concentration of cells. pH was also tested using a pH-meter which was 8,97.

The distance peak to peak (ΔE) was constant but has a value of 190mV. It was higher than expected (59mV) (Figure4). The formal potential (E^0) was +0,315V vs Ag/AgCl and the ratio between cathodic and anodic peaks was around 0,92. These results indicate slow electron transfer kinetics and reduction reaction slightly favored versus the oxidation.

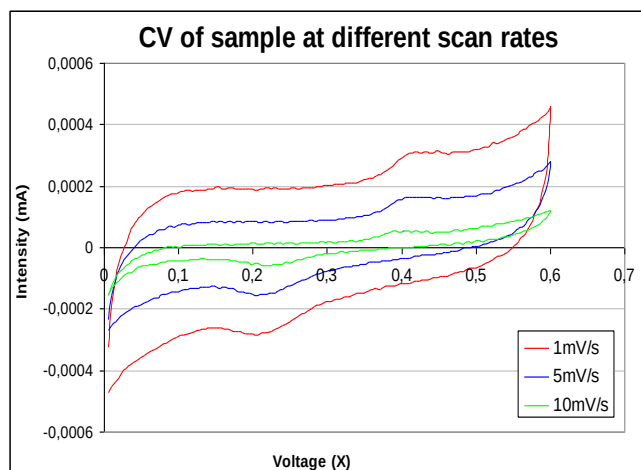


Figure4. CVs of *Anabaena* sp. sample comparing different scan rates (1mV/s, 5mV/s and 10mV/s).

The effect of the concentration of cells on the signal

In order to determine the optimum concentration of cells required for high intensity throughputs, we analyzed a variety of samples that presented different amounts of cells per milliliter and compared it with the signal that we obtained. The concentration is a parameter that directly depends on the growth state of the culture. Both the concentration and the state were studied at the same time [28]. Culture was sampled periodically, based on previous data of the group. From day zero, it takes 27 days to reach the stationary state. Samples were taken from the batch culture so that bacteria cells were in different concentrations every time. After CVs measurements were conducted, the results showed that the signal rose when 15 days passed (Figure5). The peaks intensity kept more or less constant and did not drop when the stationary state was reached.

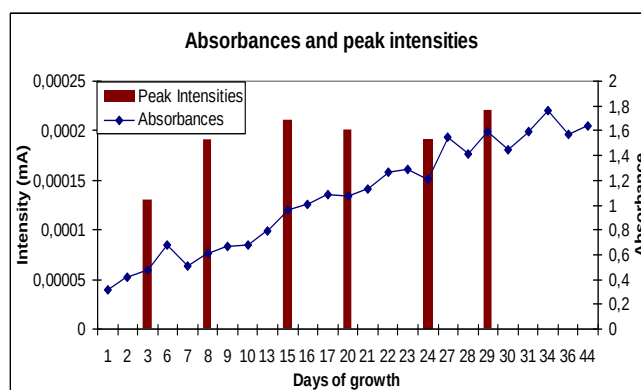


Figure5. Correlation of the anodic peak intensities (red bars) obtained with CVs at 5mV/s and the growth state of the cyanobacteria (blue line).

We concluded that, after approximately 7 days, the concentration of cells present in the sample does not affect the peak height values. From this experiment on, all samples had a minimum absorbance of 1,0 to assure that concentration did not affect the signal.

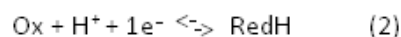
pH dependence of the formal potential

After determining the effect of concentration of cells on the electro-activity, we also wanted to study if different concentrations of protons switched the formal potential to different values.

Following the Nernst equation (1), a direct relation exists between the electrochemical potential and the concentration of the reactants [27]. A dependence of pH with the observed potential would demonstrate the implication of protons in the reaction.

Concentrated nitric acid and concentrated potassium hydroxide were used in order to modify the pH of the samples. Although the amount of the reactants added is not known, the pH could be tested using a pH-meter. The theoretical values were obtained taking the first experimental potential (+0,3048V) as the initial value and using equation (1) to calculate all the others.

$$E = E^0 - 0,0591/n \log \frac{[Ox][H^+]}{[RedH]} \quad (1)$$



pH	[H ⁺]	Ox. Peak Potential	Theoretical Values
7,87	1,34*10 ⁻⁸	0,3048	0,3048
8,43	3,7*10 ⁻⁹	0,3563	0,3378
8,75	1,77*10 ⁻⁹	0,4063	0,3567
8,97	1,07*10 ⁻⁹	0,4246	0,3696
9,31	4,89*10 ⁻¹⁰	0,4504	0,3902

Table1. Different values for pH, protons concentration, experimental oxidation peak intensities and the theoretical values calculated from Nernst equation.

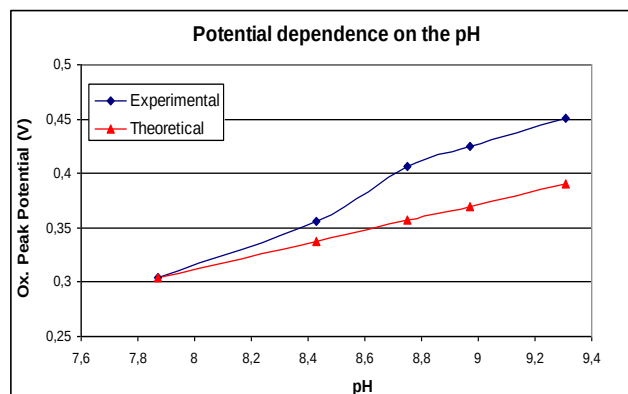


Figure 6. Experimental measurements of the oxidation peak potentials at different pH values (CVs at 5mV/s) (blue line) and the theoretical values calculated from Nernst Equation (red line).

A direct proportionality exists between the concentration of protons and the position of the peaks in the CVs (Figure6). Higher pH implies more positive potentials. However, when compared with theoretical values, the experimental data doesn't fit as expected. Experimental bias or different activity coefficients of the protons might interfere. We demonstrate the implication of protons in the system.

Separation of the culture in different phases

A centrifugation step was carried out, previous to CV, in order to separate bacteria cells from the BG-11 medium and outer molecules. If the signal appeared in the supernatant part, it could be attributed to extra-cellular components (natural mediator).

40 ml of sample were centrifuged at 5000rpm during 5 minutes with a spinning centrifuge. All the cells were deposited at the bottom of the "falcon" tube and the sample was divided in two, 20ml each. One was just made of medium

and the other was composed of medium mixed with the re-suspended cyanobacteria.

To discard that cellular components released to the medium from broken cells were responsible for the activity, another sample was taken and all the biological matter was grinded. An automatic bead-beater and zirconium/silicon pearls (0,1 to 0,5mm) were used.

The experiment demonstrates that electro-activity appears in bacteria samples but does not appear in the supernatant (Table1). In the case of grinded cells, no signal was detected whatsoever. Direct electron transfer might be the cause of electro-activity. However, further analysis is required since no evidences of this phenomenon have ever been observed.

Dialysis membrane barrier

We performed a new experiment in order to complement the data previously observed (Table2). We analyzed the possibility of direct electron transfer using a membrane as a barrier.

A dialysis membrane, 14kDa of pore, was placed between the active area of the electrode and the sample. The setting allows only those molecules smaller than 14kDa, to pass through the membrane and reach the electrode. *Anabaena sp.* cells are not able to pass through such small hole. Both demonstration of the presence of mediators and delimitation of its size, is the aim of this experiment.

CVs test were performed to 20ml of sample. A peak was obtained in the cyclic voltammetry meaning that most of the electrogenic activity observed until the moment can be attributed to extra-cellular charge carriers (Table2). No direct electron transfer is possible through the dialysis membrane.

We attributed the lack of signal in the supernatant (experiment above explained) to the centrifuging step. It was probably precipitated with the rest of the biological matter. Different times of centrifugation should be tried in future experiments.

Sample	Peak Intensity (mA)
Positive Control	0,00022
Negative Control	No peak
Precipitate	0,00017
Supernatant	No peak
Grinded sample	No peak
14kDa membrane	0,00015

Table2. Oxidation peak intensities of the CVs (5mV/s) for the different samples measured.

02. Optimization:

Light/Dark cycles.

It is well-known that light is an essential requirement for electrogenic activity in photosynthetic organisms [4]. To demonstrate the implication of the photosynthesis in the process we put the sample through different light and dark cycles.

Anabaena sp. sample was cultured under 3 hours light / 3 hours dark and CA measurements were taken periodically (every hour). The results showed a difference of 0,0017mA between light and dark conditions (Figure7). However, this behavior is only observed in the first cycle. When a second illumination period was applied, the signal did not raise again. After some repetitions, no changes were observed.

We hypothesize that this behavior is due to a possible contamination. When the samples are analyzed in the electrochemical cell, we are not able to assure sterile conditions. Heterotrophic organisms might grow in the sample perturbing the data acquired.

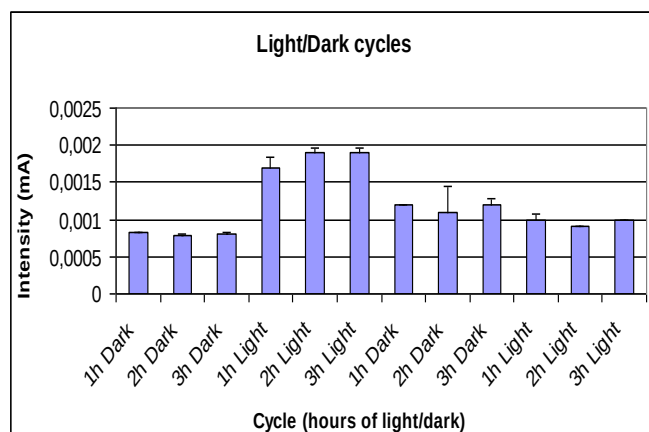


Figure7. Oxidation peak intensities of CVs (5mV/s) taken at different times under light/dark cycles.

Bubbling of the chamber

When kept in steady conditions, *Anabaena sp.* tends to aggregate in a compacted mass around the electrode. We wanted to avoid this compaction agitating the sample using a constant airflow. We introduced an air pump into the electrochemical cell and the mass transport was disturbed so that diffusion effects were no longer appreciable. Convection was predominant and a constant movement of the species was observed [27].

Cyanobacteria sample was analyzed using ChronoAmperometry (CA) and a fixed potential of +0,6V vs Ag/AgCl. The signal changed from 0,002mA to approximately 0,0025mA (Figure8). By causing a constant movement of the species, they reached the electrode at higher rates and so the intensity rose as expected.

Stress induction

Under non-optimum conditions, microorganisms attempt to adapt by changing either the environment or its metabolism [29]. Any change in their growth medium can be followed by new behavior and/or new secreted molecules. We wanted to put our sample through stressed conditions and analyze its effect on the signal.

Anabaena sp. culture was introduced in a sealed container and was left for 24 hours in steady conditions. No exchange of gases was received by the bacteria during this time.

The sample was analyzed after that period by CA and a fixed potential of +0,6V vs Ag/AgCl. The signal was the highest obtained in the optimizations, with values above 0,003mA (Figure8). The impossibility of the cells to obtain, neither carbon dioxide nor oxygen, might have made the cells produce electro-active compounds. The sort of molecules analyzed or their origin is not known. Further measurements must be performed.

Glucose as a carbon source

Previous studies of the group showed that the metabolism of either cyanobacteria or eukaryotic algae change when an external carbon source such as glucose is supplied. Since electrogenic activity is directly related with the energy generation (photosynthesis) of cells, a different energy source would theoretically diminish the signal [23].

Different concentrations of glucose (0,5% 1% 3%) were added to the medium of *Anabaena* sp. and CA analysis were conducted.

No differences between concentrations were observed (Figure8). Although the sample with 1% of glucose is slightly lower than the positive control, the result is not statistically significant (0,0005mA deviation of the values).

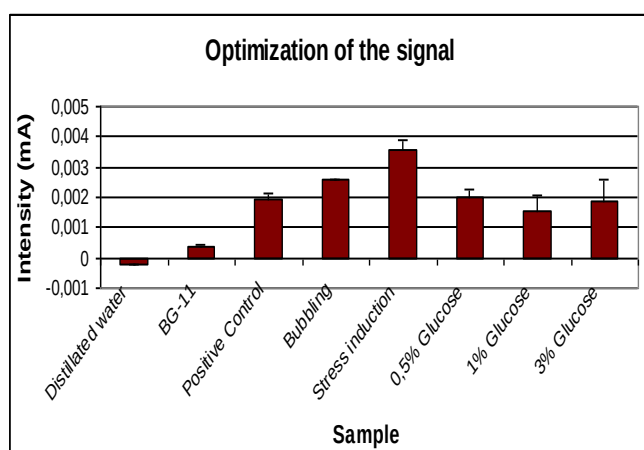


Figure8. Anodic Peak Intensities (CVs at 5mV/s) of different samples: Distillated water and BG-11 (Negative Controls), Positive Control (*Anabaena* sp.), Bubbling (cyanobacteria sample under a constant air flow), Stress induction (cyanobacteria sample after 24h left in a sealed container) and 0,5% Glucose, 1% Glucose and 3% Glucose (concentration of the glucose added).

03. Fuel Cell Construction:

We designed and constructed a two chambers fuel cell adapted to fit in both electrodes (anode and cathode) and the Proton Exchange Membrane.

The fuel cell is composed by two walls and two inner cages attached together with four screws (Figure9A). To avoid leaking issues, we placed rubber gaskets between each piece. AutoCad®

software was used for designing the prototypes (Figure9B).

PolyMethylMethacrylate (PMMA) was the material chosen because of its good light transmittance properties, its feasible machining capability and its affordable price.

For cutting purposes we draw the design on the PMMA and we used different instruments such as a power saw and a carbon dioxide laser available in our department. Sanding machine and sandpaper were used for final tweaks.

Once the structural pieces were formed (Figure9D), we placed a Proton Exchange Membrane (PEM) that splits and isolates the anode and the cathode (Figure9A). At the same time, it allows the protons generated in the metabolism of bacteria (photosynthesis) to flow from one side to the other (Figure2). A carbon fiber anode or negative electrode was placed in one chamber together with cyanobacteria while a platinum cathode or positive electrode was placed in the other chamber

There are some processes whose kinetics affect the throughput of the PMFCs and they had to be taken into account when constructing it. (i) Inside the fuel cell, cyanobacteria's metabolism generates the protons that move from one semi-chamber to the other (the distance between electrodes had to be as short as possible.). (ii) These same protons then react with the oxygen introduced by the bubbling and regenerate water (cathode is platinum due to its catalyzing properties). (iii) The electrons must be transferred from mediators to the electrode (if low transfer kinetics are measured from natural mediators, artificial ones had to be supplied).

All these factors create what is called the internal resistance of the fuel cell, and they are part of the cause of the low yields that are obtained from PMFCs [30][31]. All the reactions (i, ii and iii) are connected so the process that is rate limiting will be diminishing the velocity at which all the others are occurring.

Once all the pieces were assembled and the PMFCs was constructed, we installed a Fuel Cell

Tester in our lab (Maccor Instruments Series 4100 and MultiPlexer 4300) to carry out the battery tests (culture/discharge cycles, polarization curves, etc.)

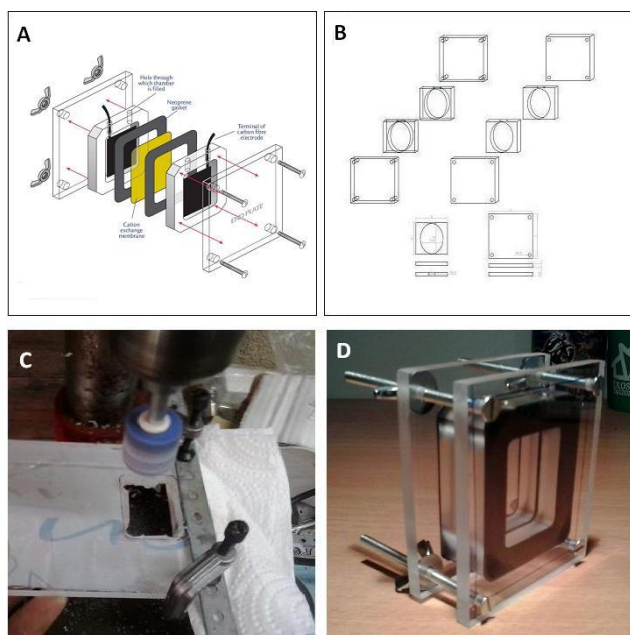


Figure 9. A. Assembling scheme of the PMFC parts B. One of the AutoCad designs prepared for construction C. While performing the fuel cell. D. The final device constructed.

CONCLUSIONS

A characterization and quantitative analysis of the interaction between the cyanobacteria *Anabaena sp.* and a graphite electrode has been reported. An extra-cellular mediator < 14kDa of size has been demonstrated to produce most of the signal obtained in CVs and protons have showed to take part in the redox reaction. The current density (current/active area) measured ($0,002\text{mA}/0,5\text{cm}^2$) was $4\mu\text{A}/\text{cm}^2$ when +0,6V (vs Ag/AgCl) were applied to the working electrode. It is 10-fold higher than the density measured in similar conditions using *Synechocystis sp.* [19]. The formal potential observed in non-modified conditions (+0,315V vs Ag/AgCl) would allow us to couple this reaction with the reduction of protons and oxygen (+1,01V vs Ag/AgCl) in a fuel cell construction. Theoretically, it would provide an electromotive force of 0,695V.

The stress induction under sealed conditions provided an increase of the intensity signal that

should be taken into account in future studies. The molecules or metabolism changes implicated could offer new strategies to raise the power of the PMFC.

Finally, a Photovoltaic Microbial Fuel Cell device was constructed in the lab as a model of electricity generator. The material used (PMMA) and the procedure followed, allow us to build low-cost fuel cells ($\approx 4\text{€}/\text{cell}$).

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REFERENCES

- [1] R. W. Bradley, P. Bombelli, S. J. L. Rowden, and C. J. Howe, "Biological photovoltaics: intra- and extra-cellular electron transport by cyanobacteria," *Biochem. Soc. Trans.*, vol. 40, no. 6, pp. 1302–7, 2012.
- [2] M. A. S. Indram, "Onsumption j," p. 2011, 2011.
- [3] S. A. Angermayr, K. J. Hellingwerf, P. Lindblad, and M. J. Teixeira de, "Energy biotechnology with cyanobacteria," *Curr. Opin. Biotechnol.*, vol. 20, no. 3, pp. 257–263, 2009.
- [4] J. M. Pisciotta, Y. Zou, and I. V. Baskakov, "Light-dependent electrogenic activity of cyanobacteria," *PLoS One*, vol. 5, no. 5, 2010.
- [5] D. R. Lovley, "Powering microbes with electricity: Direct electron transfer from electrodes to microbes," *Environ. Microbiol. Rep.*, vol. 3, no. 1, pp. 27–35, 2011.
- [6] J. S. Geelhoed, H. V. Hamelers, and A. J. Stams, "Electricity-mediated biological hydrogen production," *Curr. Opin. Microbiol.*, vol. 13, no. 3, pp. 307–315, 2010.
- [7] M. M. Ghangrekar and V. B. Shinde, "Microbial Fuel Cell : a New Approach of Wastewater Treatment With Power Generation," *Fuel Cells*, vol. 283440, no. 03222, pp. 20–21, 2006.
- [8] Z. Yongjin, J. Pisciotta, R. B. Billmyre, and I. V. Baskakov, "Photosynthetic microbial fuel cells with positive light response," *Biotechnol. Bioeng.*, vol. 104, no. 5, pp. 939–946, 2009.
- [9] T. a. Craig, F. L. Crane, P. C. Misra, and R. Barr, "Trans-plasma membrane electron transport in *Anacystis nidulans*," *Plant Sci. Lett.*, vol. 35, no. 1, pp. 11–17, 1984.

- [10] J. M. Pisciotta, Y. Zou, and I. V. Baskakov, "Role of the photosynthetic electron transfer chain in electrogenic activity of cyanobacteria," *Appl. Microbiol. Biotechnol.*, vol. 91, no. 2, pp. 377–385, 2011.
- [11] R. W. Bradley, P. Bombelli, D. J. Lea-Smith, and C. J. Howe, "Terminal oxidase mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 show increased electrogenic activity in biological photo-voltaic systems," *Phys. Chem. Chem. Phys.*, vol. 15, no. 32, pp. 13611–8, 2013.
- [12] M. Rosenbaum and U. Schröder, "Photomicrobial solar and fuel cells," *Electroanalysis*, vol. 22, no. 7–8, pp. 844–855, 2010.
- [13] G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen, and D. R. Lovley, "Extracellular electron transfer via microbial nanowires," *Nature*, vol. 435, no. 7045, pp. 1098–1101, 2005.
- [14] D. K. Newman and R. Kolter, "A role for excreted quinones in extracellular electron transfer," *Nature*, vol. 405, no. 6782, pp. 94–97, 2000.
- [15] M. Rosenbaum, Z. He, and L. T. Angenent, "Light energy to bioelectricity: Photosynthetic microbial fuel cells," *Curr. Opin. Biotechnol.*, vol. 21, no. 3, pp. 259–264, 2010.
- [16] D. R. Lovley, "Electromicrobiology," *Annu. Rev. Microbiol.*, vol. 66, no. 1, pp. 391–409, 2012.
- [17] P. Bombelli, R. W. Bradley, a M. Scott, a J. Philips, a J. McCormick, S. M. Cruz, a Anderson, K. Yunus, D. S. Bendall, P. J. Cameron, J. M. Davies, a G. Smith, C. J. Howe, and a C. Fisher, "Quantitative analysis of the factors limiting solar power transduction by *Synechocystis* sp. PCC 6803 in biological photovoltaic devices," pp. 4690–4698, 2011.
- [18] G. Kufryk, "Advances in utilizing cyanobacteria for hydrogen production," *Adv. Microbiol.*, vol. 3, no. 6, pp. 60–68, 2013.
- [19] A. Cereda, A. Hitchcock, M. D. Symes, L. Cronin, T. S. Bibby, and A. K. Jones, "A bioelectrochemical approach to characterize extracellular electron transfer by *synechocystis* sp. PCC6803," *PLoS One*, vol. 9, no. 3, pp. 1–8, 2014.
- [20] D. Singh, D. Pratap, Y. Baranwal, B. Kumar, and R. K. Chaudhary, "Microbial fuel cells : A green technology for power generation," *Library (Lond.)*, vol. 1, no. 3, pp. 128–138, 2010.
- [21] Z. He and L. T. Angenent, "Application of bacterial biocathodes in microbial fuel cells," *Electroanalysis*, vol. 18, no. 19–20, pp. 2009–2015, 2006.
- [22] R. E. Blankenship, D. M. Tiede, J. Barber, G. W. Brudvig, G. Fleming, M. Ghirardi, M. R. Gunner, W. Junge, D. M. Kramer, A. Melis, T. a Moore, C. C. Moser, D. G. Nocera, A. J. Nozik, D. R. Ort, W. W. Parson, R. C. Prince, and R. T. Sayre, "Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement.," *Science*, vol. 332, no. 6031, pp. 805–809, 2011.
- [23] T. Yagishita, S. Sawayama, K. Tsukahara, and T. Ogi, "Effects of glucose addition and light on current outputs in photosynthetic electrochemical cells using *Synechocystis* sp. PCC6714," *J. Biosci. Bioeng.*, vol. 88, no. 2, pp. 210–214, 1999.
- [24] A. J. McCormick, P. Bombelli, A. M. Scott, A. J. Philips, A. G. Smith, A. C. Fisher, and C. J. Howe, "Photosynthetic biofilms in pure culture harness solar energy in a mediatorless bio-photovoltaic cell (BPV) system," *Energy Environ. Sci.*, vol. 4, no. 11, p. 4699, 2011.
- [25] J. Shi, J. C. Claussen, E. S. McLamore, A. Ul Haque, D. Jaroch, A. R. Diggs, P. Calvo-Marzal, J. L. Rickus, and D. Marshall Porterfield, "A comparative study of enzyme immobilization strategies for multi-walled carbon nanotube glucose biosensors," *Nanotechnology*, vol. 22, no. 35, p. 355502, 2011.
- [26] D. Millo, "Spectroelectrochemical analyses of electroactive microbial biofilms," *Biochem. Soc. Trans.*, vol. 40, no. 6, pp. 1284–90, 2012.
- [27] A. J. Bard and L. R. Faulkner, *Electrochemical methods : fundamentals and applications*. 2001.
- [28] T. Yagishita, S. Sawayama, K. I. Tsukahara, and T. Ogi, "Effects of intensity of incident light and concentrations of *Synechococcus* sp. and 2-hydroxy-1,4-naphthoquinone on the current output of photosynthetic electrochemical cell," *Sol. Energy*, vol. 61, no. 5, pp. 347–353, 1997.
- [29] L. Mao and W. S. Verwoerd, "Selection of organisms for systems biology study of microbial electricity generation: a review," *Int. J. Energy Environ. Eng.*, vol. 4, no. 1, p. 17, 2013.
- [30] D. P. B. T. B. Strik, R. a. Timmers, M. Helder, K. J. J. Steinbusch, H. V. M. Hamelers, and C. J. N. Buisman, "Microbial solar cells: Applying photosynthetic and electrochemically active organisms," *Trends Biotechnol.*, vol. 29, no. 1, pp. 41–49, 2011.
- [31] M. H. Osman, a. a. Shah, and F. C. Walsh, "Recent progress and continuing challenges in bio-fuel cells. Part II: Microbial," *Biosens. Bioelectron.*, vol. 26, no. 3, pp. 953–963, 2010.