

From Spirulina to Electricity

A study on the use of the dioxygen produced by Spirulina to regenerate the catholyte of a RedOx hybrid flow cell.



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Abstract

The goal of this work is to construct a system that integrates a RedOx cell and a culture of edible algae so that the dioxygen released during their growth can be used to produce electricity. The algae that were used belong to the *Spirulina* genus and have excellent nutritional values. During the photosynthetic process, the algae take CO₂ from the Earth's atmosphere and release dioxygen. The dioxygen produced would then be used to regenerate the catholyte of a hybrid RedOx flow cell that uses zinc and methylene blue, which are non-toxic and relatively easily available materials. This type of cell is fairly new, and its potential has not yet been fully explored.

Mankind is currently struggling with what is widely described as the problem of the century: global warming. Today, it is therefore more necessary than ever to take climate change mitigation and adaptation measures. These include the use of renewable energy sources and food production that is more sustainable and respectful of our planet, with a neutral carbon footprint, such as *Spirulina*.

The experimental part of this work was subdivided into three parts, the first of which concerned the cultivation of *Spirulina*, for which the growth of the algae was studied with Zarrouk's medium. Next, the focus was on dioxygen capture, for which numerous experiments were carried out, as nothing was found in the literature. In the last part, the hybrid flow RedOx cell was realised. Here too, numerous experiments were carried out to optimise methods already found in the literature. The results obtained in the work are very promising. *Spirulina* is easy to cultivate and has a very high growth rate and dioxygen production. The dioxygen was successfully harvested using the developed method and was used to recharge the catholyte in the RedOx hybrid flow cell. In relation to this cell, the optimisation experiments carried out made it possible to use the cell to keep a small LED on for a considerable time.

To summarise, starting with a 2L culture of algae it was possible to produce 160 mL of dioxygen in 24 hours, which was enough to recharge the catholyte to keep the LED (320 Ω) on for a duration of 3 hours and 40 minutes.

Spirulina is therefore not only an important source of nutrition, but it also can be used to produce dioxygen, which was used to recharge the catholyte in the RedOx hybrid flow cell.

1. Introduction

The initial idea for this work was to study the foods of the future. The United Nations (UN), through its “Sustainable Development Goals” (SDGs)¹ programme, has defined 17 goals that mankind should achieve by 2030.² Among them, SDG number 2 aims to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture. This topic is therefore highly relevant today, and society's attention to innovative foods is beginning to grow. In recent years, more and more products with a high plant protein content have appeared on the shelves of our supermarkets. It was while doing our research in this area that we came across an article by the European Space Agency (ESA): “*Food from Spirulina experiment underway*”³. The ESA is interested in the cultivation of *Spirulina* onboard the International Space Station (ISS) as it could provide protein and dioxygen for astronauts during their missions. Being a photosynthetic cyanobacterium, *Spirulina* uses carbon dioxide in water to carry out photosynthesis, thus producing gaseous dioxygen, essential for human survival.

Using *Spirulina* as a protein source also saves numerous resources, including water. For the same mass of protein obtained, *Spirulina* crops use 1/3 as much water as soybean crops, 1/5 as much as maize crops, and only 1/50 as much water as beef protein.¹⁰ Saving and protecting water resources is vital to achieving several of the SDGs, like goals 3, 11, 13, 14, and 15, and especially Goal 6, which aims to “Ensure the availability and sustainable management of water for all [...]”¹¹. Algae are living organisms with a neutral greenhouse gas emission impact. They are able to sequester CO₂, which is only released back into the atmosphere during the direct or indirect decomposition of algae.

During a discussion with the chemistry teacher, the idea of possible further use of the dioxygen produced by algae emerged, namely using it to produce electricity. The production of electricity from renewable sources is one of the most debated topics these days. The seventh goal of the UN's SDGs relates precisely to this topic: “Ensure access to affordable, reliable, sustainable and modern energy for all”¹². This led to the idea of building a RedOx flow cell, a rather new system whose potential has not yet been fully explored. The idea from where this project started was to exploit algae cultivation both to obtain food, and to recover the dioxygen produced to recharge a hybrid electrochemical flow cell.

1.1 Spirulina

Spirulina (Arthrospira) belongs to the oxygenated photosynthetic bacteria that cover the Cyanobacteria and Prochlorals groups.^{4,5} It is a cyanobacterium that forms colonies of several individuals and is characteristically spiral-shaped.⁶ They are generally found in tropical and subtropical regions in warm bodies of water with high carbonate/bicarbonate content and high pH and salinity.⁶ *Spirulina* was one of the first organisms on earth to acquire the ability to carry out photosynthesis and is thought to be the organism from which many of the plants we know today are derived.⁷ It has been present on our planet for 3.5 billion years and more specifically has been growing wild for millions of years in Africa and South America.⁸ In many African countries, it is used by humans as food because of its high protein content: it is harvested from natural water, dried and then eaten.⁹ The resulting powder is often added to pasta, bread, and other baked goods, but also condiments, chips, cheese, chocolate, ice cream, candy and honey. *Spirulina* is also added to many drinks.⁸

1.2 RedOx ibrid flow cell

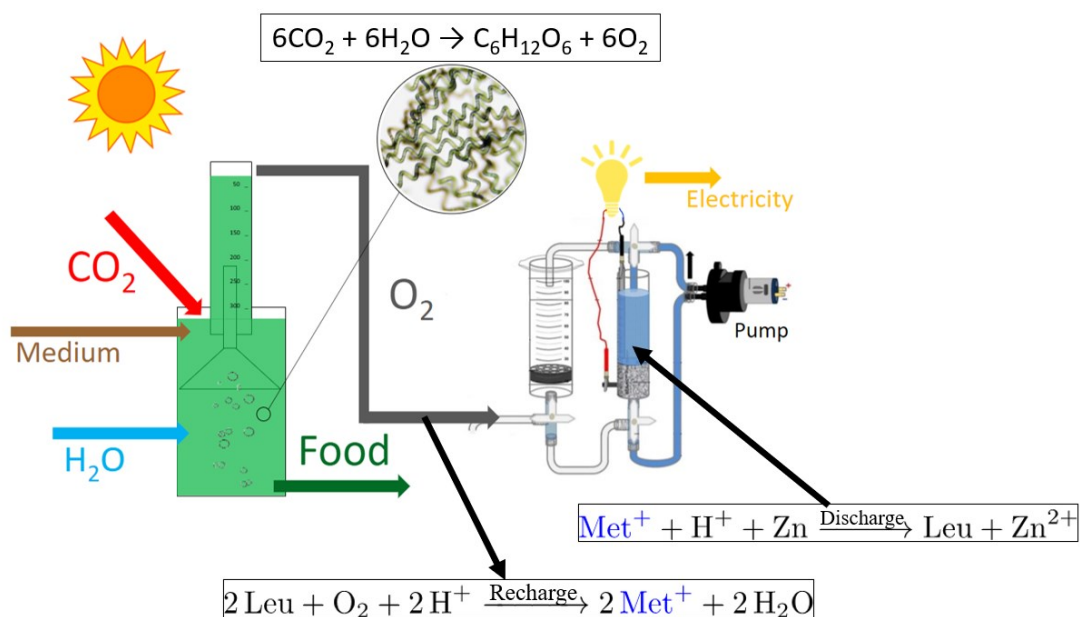
The type of cell that we studied is referred to as a hybrid RedOx flow cell and is characterised by one of the two active substances not being present in solution (aqueous or gaseous), but in solid form (e.g. in the form of a metal electrode). The presence of a solid electrode allows the cell to be operated in a single reaction chamber, eliminating the need to use an extremely expensive selective semi-permeable membrane, which would be otherwise needed. The second electrode usually consists of an inert material that has a good affinity for the active substance in the solution. However, metal electrodes are not a good solution for use as secondary electrodes, as they are easily degraded and produce oxides that lead to their progressive electrical inactivity. Preference is therefore given to the use of special graphite sponges, made of a very porous material, which has a large exchange surface area, thus favouring the flow of electrons and consequently a high reaction rate.¹³

The use of methylene blue and metallic zinc as active substances in our cell enables the creation of a battery with improved environmental performance. Very often, the problem with other batteries arises with their disposal, as they are often abandoned in open dumps in developing countries, causing considerable damage to the local environment. The substances contained in these batteries are released uncontrolled into the environment and can enter the water table, contaminating entire ecosystems.^{14,15}

1.3 Summary of the proof of concept

As it is shown in the diagram below (**Figure 1**), cultivating *Spirulina* not only enables food production but also energy production. The specific study of *Spirulina*'s living conditions and the optimisation of cultivation allows, in addition to increased growth and consequently increased food production, increased production of dioxygen. The cyanobacterium performs the photosynthesis reaction, which produces O₂ and glucose from CO₂ and H₂O. It is precisely the dioxygen produced by this reaction that is taken up and fed into a circuit to be used to recharge the spent catholyte in the RedOx flow cell. The cell, once put into operation, allows electricity to be produced. The cultivation of a simple photosynthetic cyanobacterium thus produces food and electricity.

Figure 1: The system as a whole



2. Experimental

For the system to work properly, it is first necessary to obtain a stable algae culture with a good dioxygen production rate. Then, it is necessary to proceed with the collection of the biomass of algae growth, and then convey it to the tank where the catholyte must be refilled. To this end, three main fields of investigation have been identified: cultivation of *Spirulina* (2.1), collection of the dioxygen produced by it (2.2) and construction of the cell (2.3) The methodology

described here was developed by us, taking existing literature as a basis. Thanks to this work, it was possible to develop a reliable procedure that enables the creation of the complete system.

2.1 Culturing *Spirulina* with Zarrouk's medium

The very first thing studied in the work was the method of culturing *Spirulina*. The culture was started according to the procedure described in a special brochure from the supplier company¹⁶. Once the culture was started, filtration and separation experiments were carried out to collect the edible algal biomass and start a new culture. Having run out of salts available for *Spirulina* growth, it was decided to produce a culture medium in the laboratory that would allow the growth of new cultures. After evaluating several possible recipes for the preparation of these mediums, Zarrouk's medium¹⁷ was chosen.

2.1.1 Materials

- Salt for 10 L from Zarrouk's medium
- 10 sheets of paper
- Becker 50 mL
- Aquarium
- 10 L bin
- 400 mL of *Spirulina* culture
- 10 spatulas
- Libra
- Analytical balance
- 2 magnetic stirrers
- Becker 1500 mL
- Deionised water

2.1.2 Methodology

Using a different spatula for each salt (**Figure 2.2**), the required mass was measured with a scale. A piece of paper was placed on the scale plate on which each salt was weighed. All were subsequently combined in a beaker. 10 L of culture medium was prepared. The salts were dissolved in a beaker placed over a magnetic stirrer (**Figure 2.1** and **Figure 2.3**), after which they were decanted into a 10 L tank.

To start this culture, a 1,500 mL beaker was taken and placed on top of a magnetic stirrer near the other cultures, in front of the window. 400 mL was then taken from the initial *Spirulina* culture to which 600 mL of the solution containing the various salts was added (**Figure 2.4**). This was then brought to volume with deionised water. The beaker with the magnetic stirrer was then placed in front of a window, in a position avoiding direct sunlight. The magnetic stirrer was set to ~ 250 rpm and was connected to a timer for switching it on, which occurred for half an hour at 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00. It was subsequently necessary

to check the culture at least once a week to ensure that not too much water had evaporated. In this case, deionised water had to be added to compensate for the evaporated volume.

Figure 2: Starting a new crop with Zarrouk's medium

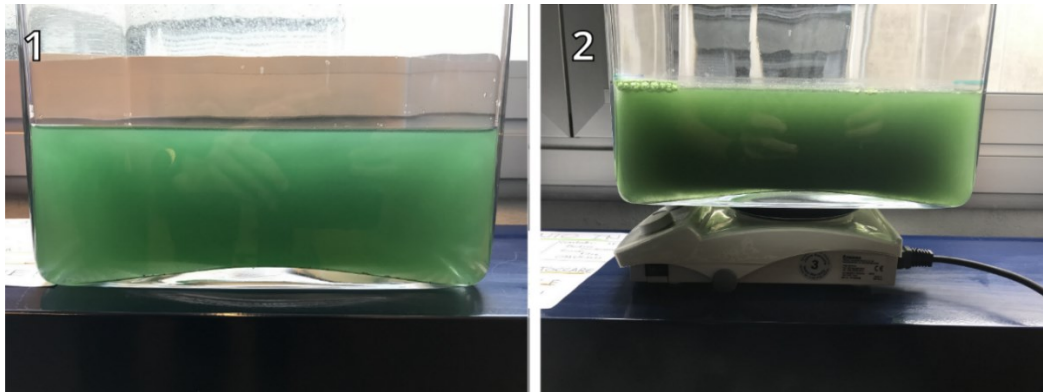


1. Deionised water in the aquarium above the magnetic stirrer before adding the salts. 2. Salts used. 3. Aquarium with deionised water placed on top of the magnetic stirrer immediately after the addition of the salts. 4. Becker containing the first culture started with Zarrouk's medium.

2.1.3 Results

In the days after planting, the colour of the culture changed noticeably, becoming much darker, as it can be seen in *Figure 3.1* taken on 11 May (culture inoculation) and *Figure 3.2*, dated 17 May 2021.

Figure 3: Comparison of culture at 6-day intervals



1. Aquarium with the second culture started with Zarrouk's medium. 2. Aquarium with the second culture started with Zarrouk's medium after 6 days.

2.2 Capture of dioxygen

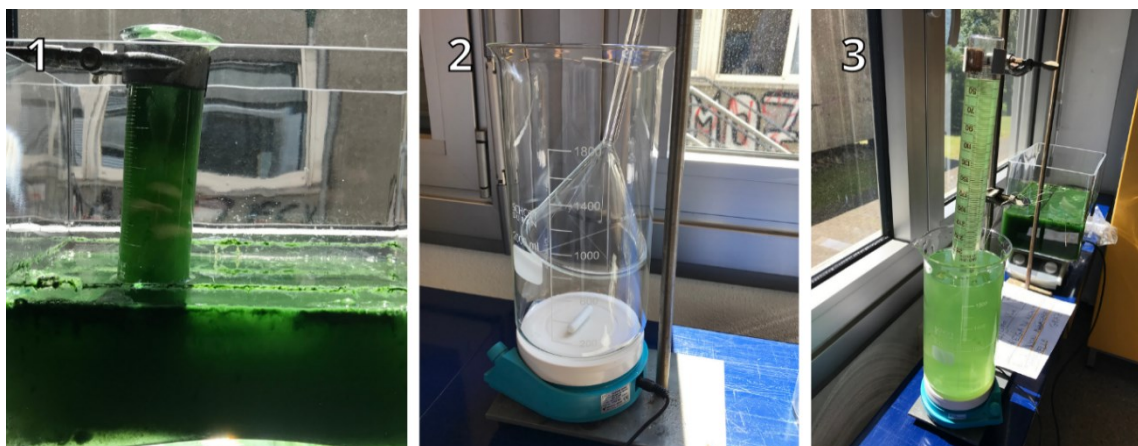
Having improved our knowledge of *Spirulina*, we now look for ways to capture the dioxygen produced by it. This chapter will therefore explain the procedure adopted to achieve this.

2.2.1 Materials

- Becker high and narrow (1800 mL)
- *Spirulina* culture
- Growing medium
- Clamp
- Fishing line
- Magnetic stirrer
- Deionised water
- Stand
- Glass funnel
- Graduated cylinder

2.2.2 Methodology

At the top of the beaker containing the new culture, an inverted funnel was placed to cover almost the entire water column, held up with the fishing line and then tied to the clamp of the stand (**Figure 4.2**). Above the end of the funnel was placed a graduated cylinder, filled with *Spirulina* and with the opening submerged in the culture, supported by a stand (**Figure 4.3**).

Figure 4: Methods for capturing dioxygen

1. First method of capturing dioxygen with cylinder immersed in the culture. 2. Method of holding up the funnel for the second attempt to capture dioxygen. 3. Second and final attempt to collect dioxygen.

2.2.3 Results

This method allowed us to collect 160 mL of dioxygen (measured under normal conditions of pressure and temperature), over approximately 72 hours (Figure 5).

2.3 Construction of the RedOx hybrid flow cell with LEDs

Numerous attempts have been made (of which the description is omitted in this document) to constrain the RedOx hybrid flow cell, inspired in particular by the work of Quarthal et al.¹⁸ The best of these, in which even a small LED could be switched on, is described below.

To enable the cell to function, in addition to replacing the graphite electrode, experiments had to be carried out on the pH value at which the reaction was to take place.

In the first attempts, it was noted that the reaction was very slow, but an increase in the acidity of the solution made it possible to speed up the reaction. The last attempt carried out and described below was the final construction of two elements, then connected in series, on which some measurements were carried out during normal operation cycles with a connected LED.

Figure 5: Captured dioxygen

The red lines help visualise the lowering of the culture level in the cylinder due to the production of dioxygen.

2.3.1 Materials for the construction of the LED cell

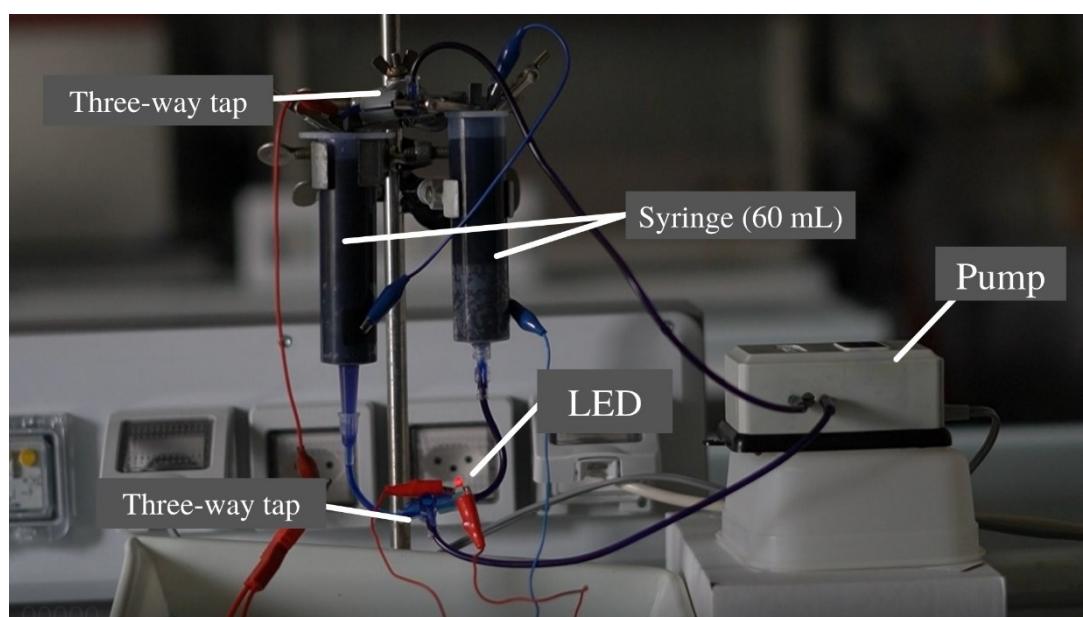
- 2 Graphite felts as electrodes¹⁹
- Cables with crocodiles clips
- Plastic tray
- Stand
- 2 Three-way taps (Discofix[®] C, B. Braun)
- Infusion set - CODAN[®]
- 200 mL flask
- Methylene blue
- LED
- PASCO[®] Current Sensor²⁰
- 2 PASCO[®] Interface²¹
- Awl
- Spark plug
- Zinc sulphate heptahydrate (ZnSO₄.7H₂O)
- 2 Mines of a graphite pencil
- 2 Clamps
- 4 Zinc electrodes
- Pump (0.14 L/min)
- 2 Syringes (60 mL, Omnifix[®], B. Braun)
- Flask (200 mL) with cap
- Deionised water
- Connection Cables
- PASCO[®] Voltage Sensor²²
- Computer with PASCO[®] Capstone software²³
- Lighter
- Sulphuric acid (H₂SO₄) C%, m/m = 98%.
- Pipette
- Aquarium aerator
- Syringe (100 mL, Omnifix[®], B. Braun)

2.3.2 Methodology

Two 60 mL syringes without needles were used; they were placed on top of a tray to avoid soiling the work table and held by a stand. A hole was drilled in both syringes for the insertion of the graphite pencil lead with a heated awl over a candle, taking great care to drill it the exact size of the lead. At the lower ends of the two syringes, two small tubes of an infusion set (CODAN[®]) were connected to a three-way stopcock, which in turn was connected to a pump. On the side from which the liquid is pumped, another small tube identical to the one used previously was placed, which was then connected to a three-way stopcock that runs via two small tubes into the two syringes. Two zinc electrodes (held slightly apart) were also placed in each cell at a distance of approximately 0.7 cm from the upper edge of the graphite felt rolled upon itself (4 x 16 cm). The electrodes were attached to the syringe by means of clamps. The two cells were connected in series: the graphite electrode of the first cell was connected to the double zinc electrode of the second cell. The measure circuit is then placed between the graphite electrode of the second cell and the double zinc electrode of the first cell. The LED was connected to one of these poles (attention was paid to the polarity of the two ends), the current

sensor was then connected in series. Via two cables with alligator clips, the voltage sensor was connected to the two opposite ends of the measuring circuit. Both sensors were connected to two interfaces, which in turn were connected via USB to a PC equipped with Capstone software (PASCO). After launching the software and pairing the sensors, data acquisition could proceed. A value of 1 Hz (one measurement per second) was chosen as the sampling rate. The structure of the final system is shown in *Figure 6*.

Figure 6: RedOx cell with LED and measuring system



For the operation of the cell, a methylene blue solution (called catholyte) was prepared: 0.036 g of methylene blue and 34.5 g of zinc sulphate heptahydrate were placed in a 100 mL flask. These were dissolved in deionised water and then the solution was increased to a volume of 120 mL, again with deionised water. Finally, a few drops of sulphuric acid were added to the solution. The catholyte was discharged separately and the resulting methylene blue solution was placed in the 200 mL flask. Through the stopper in which two holes were drilled, an aerator connected to a small tube was inserted on one side and a small tube connected to a tap on the other. The flask was then carefully capped. In the dioxygen collection system described in Chapter 4.2, a small tube was inserted into the inverted cylinder, so that a syringe could be used to collect the product. The dioxygen is collected in two 100 mL syringes. The syringe is then

connected to the aerator and with the tap closed, the syringe is emptied (**Figure 7**). By proceeding with small injected volumes, frequently shaking the flask containing the solution, and venting when the pressure reached is too high, the catholyte can be recharged. The resulting solution can then be divided between the two syringes, the pump started and measurements can begin.

2.3.3 Results

Thanks to the improvements already made to the cell, the presence of two cells in series and the increase in the surface area of the zinc electrodes, it was possible to achieve voltage and current values that allowed the LED to be switched on: it remained on for 3 hours and 40 minutes.

Concerning the measurements taken, it was possible to simultaneously measure the current and voltage between the two poles of the double cell as a function of time using the probes. For the sake of simplicity, the data from all the measurements taken were exported into two charts (**Figure 8** and **Figure 9**). The abscissa shows the voltage (in millivolts) respectively the current (in milliamperes), and the ordinate the time (in seconds). Calculated regression lines were fitted using a sixth-degree polynomial approximation.

Figure 7: Charging the catholyte with dioxygen from the culture

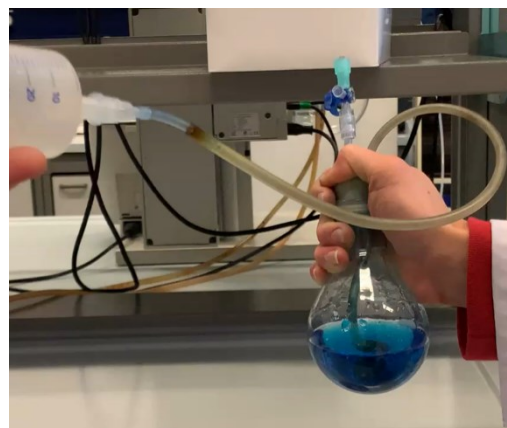
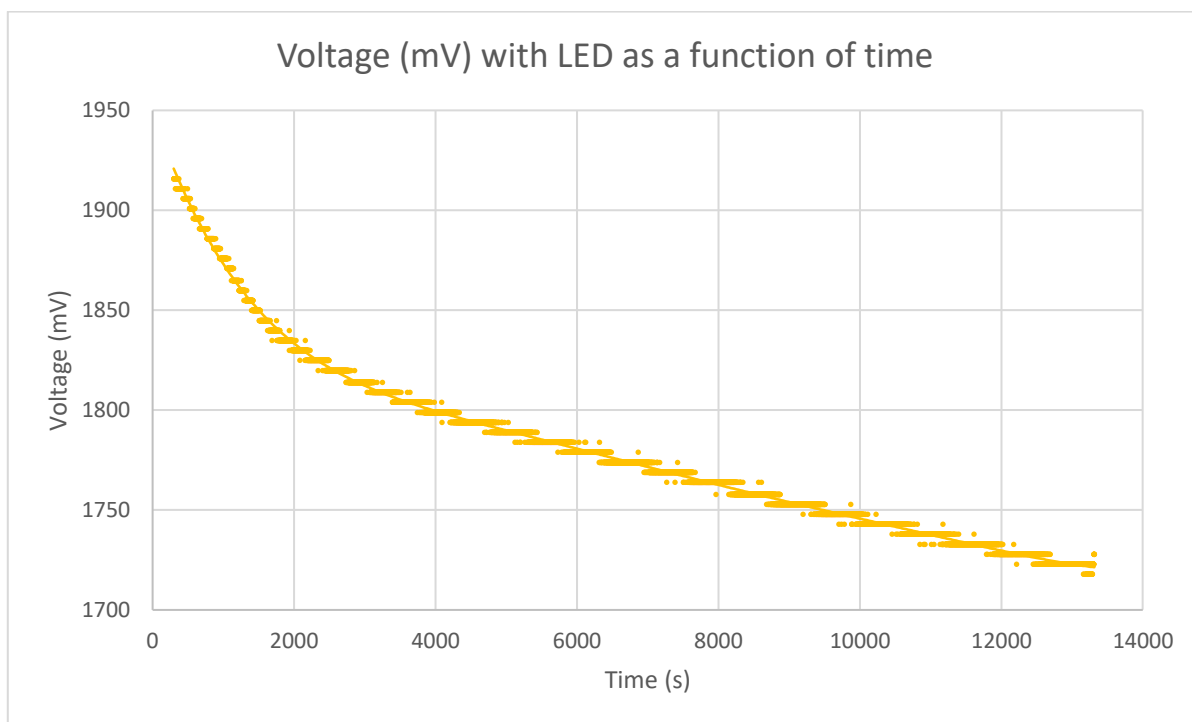
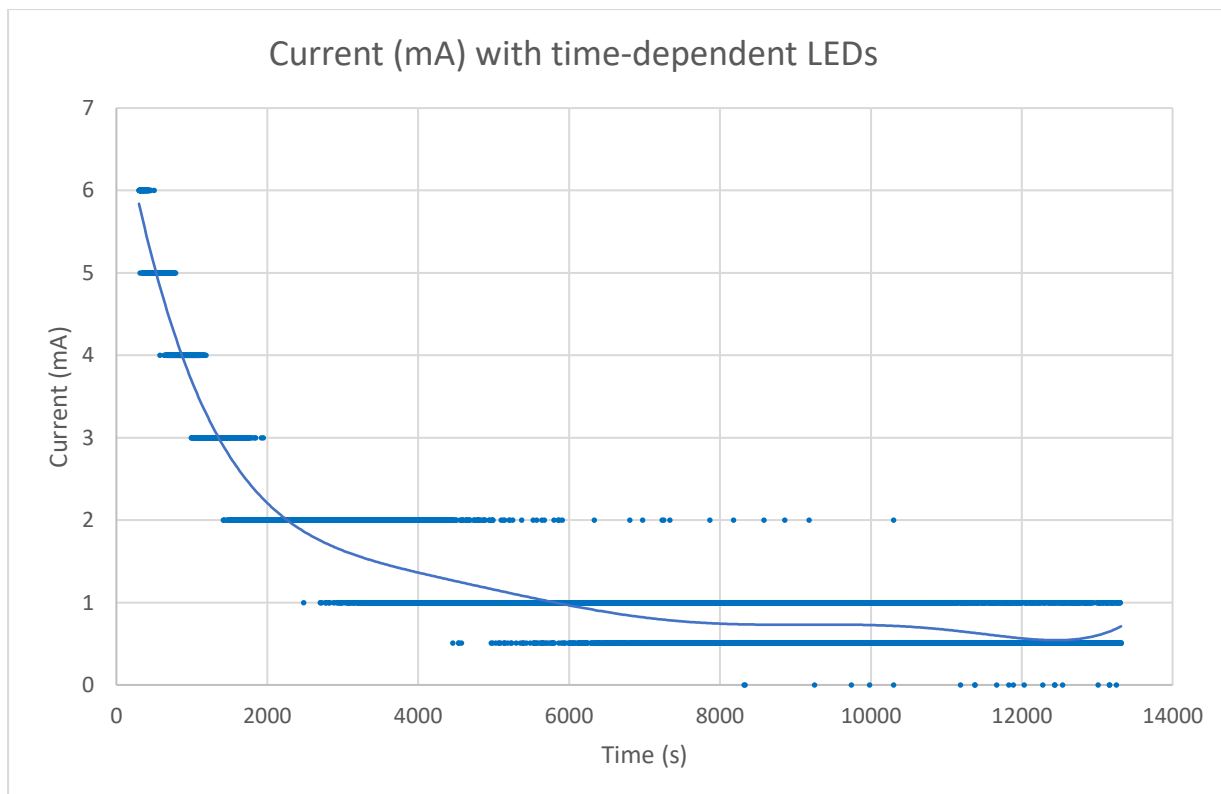


Figure 8: Graph of voltage (mV) as a function of time**Figure 9:** Graph of current (mA) versus time

2.4 Discussion of results

There are many factors influencing the growth of *Spirulina*, such as temperature, light, frequency of stirring, and composition of the medium. The parameters chosen resulted in good growth rates for the purpose of this study to obtain the *Spirulina*, but also the dioxygen produced. Further investigations to determine which parameters influence the growth of *Spirulina* and its dioxygen production could be carried out as a continuation of this research.

After initial unsuccessful attempts, the method to capture the dioxygen described above led to results that exceeded expectations: it was possible to collect a significant amount of dioxygen (160 mL) in a relatively short time (24 hours). It was also noted that with the addition of a light source even during the night, dioxygen production increased. This little stratagem also made it possible to achieve the amount of dioxygen required to recharge the catholyte of the RedOx hybrid flow cell.

Finally, the recharged catholyte and the voltage increase achieved by doubling the cell made it possible to keep the LED on for a considerable time. Using the precise measurements taken between the probes and the subsequent processing of the data with Excel, it was possible to carry out a calculation of the generated electric current (integral calculation). To do this, the value of the instantaneous power was calculated ($P = U \cdot I$), which is considered constant over one second: given a large number of data, this can be considered a very good approximation. We then proceeded to add up all the instantaneous power values, multiplied by their respective Δt (in the case considered always equal to 1 second). The electrical energy delivered is thus 31.7 joules.

3. Conclusions

Spirulina is a very easy algae to cultivate and produces a lot of biomass in a relatively short period, according to literature. Its excellent nutritional qualities make *Spirulina* a very interesting food. In a future in which mankind will have to adapt to sustainable production, which on the other hand allows us to feed an ever-increasing population, it will be necessary to pay attention to this type of high-protein, but low environmental impact food.

A further advantage of using *Spirulina* as a protein source is related to the emission of greenhouse gases. An algal culture such as the one used has in fact a low impact on the environment: cyanobacteria take CO₂ from the atmosphere and sequester it for the production of organic matter, which can then be consumed. Thus, there is no new gas input into the atmosphere, but a closed and continuous cycle is maintained.

The need to replace fossil fuels with renewable energy sources presents mankind with many technical challenges. The development of batteries that allow large amounts of energy to be stored in a short time is therefore of vital importance. It was very interesting to develop a cell that would allow recharging through the use of the dioxygen produced by the algal culture. A further advantage of such a cell is the possibility of simply and efficiently storing the catholyte once it has been recharged and is thus ready to be used in the cell: as it is a fluid, it only needs to be transferred (by means of a pump) to a separate reservoir and, once it is required, it simply needs to flow into the reaction chamber. Through the construction of larger systems combining several cells, it should then be possible to increase the volume of the storable catholyte and thus increase the storage capacity of the cell as well as the electrical power delivered by the system.

The result obtained by lighting the LED for 3 hours and 40 minutes allowed us to monitor the functioning of the system. This achievement gave us great satisfaction.

To conclude, allow us to reflect on water, a fundamental substance for Life and an increasingly precious commodity that deserves respect and attention from all of us. From this point of view, the proposed system presents interesting advantages and perspectives. *Spirulina* culture in fact requires reduced quantities of water compared to those needed for other agricultural practices, and one of its waste products, dioxygen, can be recovered to produce electricity in a sustainable and environmentally friendly way.

4. Future developments

4.1 *Spirulina*

As far as *Spirulina* is concerned, the influence of each culture parameter should certainly be studied in more detail, as they have a direct impact on the biomass produced, but also on the production of dioxygen. One should therefore hopefully determine the best conditions under which *Spirulina* grows and produces dioxygen.

From the point of view of dioxygen collection, on the other hand, the dioxygen collection and capture system should be studied in more detail and can be optimised. One advantage could be the use of larger cultures in special containers, for example. It would also be necessary to find a scientifically accurate method of quantifying the dioxygen produced by the algae (e.g. taking into account the pressure in the capture chamber and the actual purity of the captured dioxygen).

4.2 RedOx

With regard to the study of the reduction reaction of methylene blue, the question of the best pH value at which the reaction takes place in the shortest time should be further investigated. With regard to the oxidation reaction of leucine methylene blue, the question of whether it would be advantageous to use a lot of dioxygen in a short time (although the results we have obtained would answer this question in the negative) or whether it would be more appropriate to leave a constant low flow of dioxygen so that the reaction takes place without waste, but more slowly, should be investigated further.

It would also be interesting to make the whole-cell system as efficient as possible, for example by quantifying the internal resistance and devising a way to minimise it. It would also be interesting to be able to have two cells and have the two cycles take place at the same time, but staggered so that one cell is always able to supply current during the charging time of the other.

4.3 Complete system

The complete system was operated with the double cell ([2.3](#)), so it is demonstrated that the system can be scaled. However, it was not possible to construct a self regulated system that would allow the automatic transfer of dioxygen from the collection system to the second part of the cell. This could, for example, be implemented by means of a mechanical syringe.

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