Mestrado Integrado em Engenharia Química

Reactor design for a family production of *Spirulina spp*. and parameters determination for a *Spirulina spp*. culture

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Abstract

Spirulina spp. is a microalga rich in proteins (55% to 65%), with nutritional and therapeutic properties. This work has the objective of designing a family photobioreactor and assesses the parameters for *Spirulina platensis* cultures.

The design of this reactor will allow the consumption of *Spirulina spp.* without loss of their important nutrients, and get it cheaper. For that, a study has began to cursing on the functions that it should carry out, as well as the necessary criteria, the geometry, the agitation and light system, to use in the future photobioreactor.

The parameters determination of *Spirulina platensis* culture was accomplished by the determination of the biomass concentration using two fundamental methods, with a constant temperature and pH, at different light intensities. The first method consists in measuring the absorbance of this microalgae, at a wavelength of 750 nm. The second method is aimed at drying the biomass at 110°C, after the rinsing and filtration of a sample withdrawn from the photobioreactor implemented in laboratory LAGEP. This study allowed verifying the influence of the light intensity in the *Spirulina platensis* development.

For reactor conception the results are in development, but it was possible to obtain four solutions for the future photobioreactor. The *Spirulina platensis* experiences realized prove that the light intensity have an important rule in the culture growth, consequently in the productivity and in growth rate. The same can be proved for the blue pigment, Phycocyanin.

Keywords: *Spirulina platensis,* Culture, Design, Photobioreactor

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Notation and Glossary

a	Apothem of truncated cone	m
DT	Density	g/l
$D_{1\ int}$	Cylinder intern diameter	m
$D_{2\ int}$	PVC tube intern diameter	m
D_{20}	Density a 20ºC	g/l
$C_{CO_3^{2-}}$	Carbonate concentration	g/l
C_{final}	Final concentration	g/l
C_{HCl}	Hydrochloric acid concentration	g/l
$C_{HCO_3^-}$	Hydrogenocarbonate concentration	g/l
$C_{initial}$	Initial concentration	g/l
C_{inorg}	Total inorganic carbon concentration	g/l
$C-PC$	Phycocyanin concentration	g/l
C_x	Biomass concentration	mg/ml
h_t	Truncated cone height	m
h_1	Cylinder illuminate height	m
h_2	Cylinder height	m
P	Productivity	g/ l/ day
Q_{max}	Maximum air flow	m^3/s
Q_{min}	Minimum air flow	m^3/s
$R_{1\ int}$	Cylinder internal radius	m
r_t	Radius lowers of the truncated cone	m
R_t	Radius upper of the truncated cone	m
T	Temperature	ºC
V_{sample}	Volume of *Spirulina platensis* sample	ml
V_1, V_2	Volume added of HCl	ml

Greek Letters

μ_{max}	Maximum specific growth rate	s^{-1}
Δt	Time interval	days

Index

$C_1,...,C_{10}$	Criteria
$F_1,...,F_{10}$	Function
i	Counter
n	Counter

Acronyms list

APTE	Application of Techniques of Enterprise
DO	Optical Density
FSD	Functional Specifications Document
GRAFCET	Graphe Fonctionnel de Commande, Etapes Transitions
SADT	Structured Analysis and Design Technique
UV/VIS	Ultraviolet/ Visible

Introduction

Spirulina platensis is a photosynthetic microorganism, which converts CO_2 into oxygen, existing on Earth 3500 million years ago. It is rich in protein with 50 to 70% db (dry basis) on vitamins (vitamin B_{12}, vitamin A, β-carotene ...), and in all essential fatty acids (linoleic acid and γ-linolenic acid). The cell walls formation of this microalgae and the absence of cellulose facilitate its digestion. Due to its characteristics it presents a very significant nutritional value, therefore, it can have multiple applications at nutritional and therapeutic levels *(Peiretti et al., 2008).*

For all those characteristics, *Spirulina spp.* cultures have been studied especially for the influence of one culture parameter (pH, light or minerals) and some follow up cultures have been presented to various photobioreactor designs.

Thus, this study has two objectives: the first was to evaluate the performance of the photobioreactor of the laboratory LAGEP. The reactor was characterized with influences of culture evolution to get experimental values to be simulated and optimized. That allows determining its limits and productivity in function of light power and temperature. And also the maximal phycocyanin concentration that is possible to be obtained. That was made through the culture evolution study which took approximately 20 days for each culture.

A reactor design study has been done to give an orientation for a familial *Spirulina spp.* reactor, as Li proposed one **for the daily life of a family** *(Li et al., 2004)*. In fact, many persons ask for means to begin a culture of *Spirulina spp.* to their own consumption or to try to create a production (instead 3 farms 10 years ago, there are now in France around 20 farms producing *Spirulina spp.*). On those grounds are the exponential world market of this microorganism, its high price for the consumer and the difference between the taste of fresh and dried *Spirulina spp.*.

This work is divided into three parts: the first one is the literature research carried out for *Spirulina platensis*, the second is the design of the reactor and the third is the study of the culture evolution.

Chapter I: Bibliography of *Spirulina platensis*

1. History

Spirulina spp. is a primitive body with origin dating back to 3.5 billion years and able to use the carbon dioxide dissolved in seawater as a source of nutrients for their breeding *(Habib et al., 2008).*

The knowledge of this body took place in the sixteenth century, when the Spaniards conquered Mexico. They found that the Aztèques in Mexico collected a "new food" from the lake of the Valley of Mexico. It was a kind of blue mud of high nutritional value, called *Tecuitlalt.*

In 1940, this microorganism was rediscovered by a french Phycologist, P. Dangeard, when he mentioned the consumption of cakes, Dihé *(Dangeard, 1940).* Dangeard studied samples of Dihé and concluded that it was "a pure true of a spiral-shaped blue filamentous algae". The alga was *Asthrospira platensis (Ciferri, 1983).*

Currently, *Spirulina spp.* is produced and consumed in several countries (Germany, France, Philippines, India, Africa, USA, …) *(Henrikson, 1994).*

2. Classification and Taxonomy

The *Spirulina spp.* is a prokaryotic photosynthetic be and classified as Cyanobacterium. This microorganism belongs to the category of photosynthetic bacteria that produces oxygen *(Castenholz and Waterbury, 1989; Whitton, 1992).*

Cyanobacteria, the family in which *Spirulina spp.* is included is a Gram-negative bacteria (nature of cell walls) and performs the same type of photosynthesis that occurs in plants *(Lindblad et al, 1998).*

The microbiologists, put this group of microorganisms within the class of bacteria, since both are prokaryotic cells, Gram-negative, and both have the same results at the biochemical and physiological tests *(Castenholz and Waterbury, 1989).* However, microalgae such *Spirulina spp.* have their own characteristics that allow distinguishing themselves from most bacteria. Those characteristics are:

a) The principal photosynthetic pigment is chlorophyll a, while in most bacteria it is bacteriochlorophyll;
b) The photosynthesis accomplished by the algae produces oxygen, unlike other bacteria;
c) The size of cyanobacteria is 5 to 10 times higher than that of bacteria

Spirulina spp. belongs to the subgroup of filamentous cyanobacteria, the cyanophytes, which

are characterized by the absence of mitochondria, chloroplasts, Golgi apparatus and endoplasmic reticulum. This group is distinguished from bacteria by the presence of chlorophyll a and water soluble pigments such as red (phycoerthrin) and blue (phycocyanin), as well as phycobilin. Besides, they also have carotenoides: β-carotene, echineon, zeaxanthin, myxoxanthophyll. But the most important characteristic of the cyanophytes is the easy adaptation in different resources (fresh water, water of the sea and even in the terrestrial ecosystem).

The cyanobacteria are classified in five groups. Thus, *Spirulina spp.* belongs to the kingdom Monera. This specie belongs to the sub-class of Hormogonophycidees, which is in the class Cyanophyceae. This specie is included in the Nostocales order and Oscillatoriaceae family.

3. Morphology

Spirulina platensis is a unicellular being, distinguished by its non-branched and helical filaments form, with a length of 200-300 µm, and 5-10µm width (*Chronakis et al., 2000*). According to the source, *Spirulina platensis* has three different forms: spiral, straight and wavy.

4. Biochemical composition

Spirulina spp. biochemical composition reflects its potential in the food and feed area, as well as in therapy. This varies depending on the culture conditions, which causes a large difference in the proteins values.

Table 1 - Composition of the *Spirulina* (*Clément, 1975; Ciferri, 1983; Fox, 1986; Cornet et al., 1992*)

Composition	Percentage (%)
Protein	50 – 70
Essential fatty acids	5.6 – 7
Nucleic acid	4 – 5
Carbohydrates	13.6
Ash	6.4 - 9

By elemental analysis of *Spirulina platensis* dry biomass was possible to obtain elemental composition *(Cornet et al., 1992):*

$$C_{1,58}O_{0,46}N_{0,17}S_{0,007}P_{0,006}$$

4.1. Protein

Spirulina spp. is known for its richness in proteins, due to its exceptional value when compared with other microorganisms *(Bujard et al., 1970)*. The values will depend on the source of origin and time of collection, however, within a range between 50-70% *(Habib et al., 2008)*. Most of the proteins from plants have lower values (about half) compared to *Spirulina spp. (Falquet and Hurni, 2006)*. Thus, the proteins in this microorganism have the advantage of being easily assimilated by the body *(Bujard et al., 1970)*.

From a qualitative point of view, the essential amino acids represent 47% of the total protein weight, which makes the *Spirulina spp.* complete from a protein point a view *(Bujard et al., 1970)*.

4.2. Essential fatty acids

The total lipid from *Spirulina spp.* is approximately 5.6 to 7%. But sometimes it gets to 11%, depending on the extraction system used *(Elyah, 2003)*. These lipids are divided into two parts: the saponifiable (83%) and unsaponifiables (17%).

Spirulina spp. is rich in γ-linolenic acid (omega-3), linoleic acid (omega-6), stearidonic acid (omega-3), cicosapentaenoic acid (omega-3), arachidonic acid (omega-6) *(Habib et al., 2008)*.
These acids are known for their medicinal properties, such as chemical mediators in inflammatory and immune reactions, as well as its role in many metabolic processes *(Venkataraman, 1998)*.

Spirulina spp. can be considered as a source of γ-linolenic acid, after the human milk and some vegetable oils of little use *(Ciferri, 1983)*.

4.3. Nucleic Acid

The content of nucleic acids (DNA and RNA) is important in the level food nutrition because of the biochemical degradation of some components (purine, adenine and guanine), that can produce uric acid and consequently can cause kidney stones and gout crisis *(Boudéne et al., 1975)*.

Spirulina spp. dry biomass has 4-5% of nucleic acids. Regarding the proportion of DNA and RNA, it presents lower values compared to most of the microalgae, 0.6 to 1% and 2.2 to 3.5%, respectively *(Ciferri, 1983)*.

4.4. Carbohydrates

Spirulina spp. has about 13.6% of carbohydrates in its dry biomass. Most carbohydrates are glucosamine (1.9%) and rhamnose (9.7%) or glycogen (0.5%). There are, also, in small quantities, glucose, fructose and saccharose; as well as glycerol, mannitol and sorbitol.

As mentioned before, the cellular walls of *Spirulina spp.* are similar to those of Gram-negative

bacteria, since these are composed by glucosamine and muramic acid, which are associated to the peptides. Thus, these walls are relatively weak, making its cellular contents accessible to the digestive enzymes, being an advantage over other microorganisms *(Falquet and Hurni, 2006).*

From the nutritional point of view, the meso-inositol phosphate is a substance of significant importance in *Spirulina spp.*, because it is an excellent source of organic phosphorus and inositol *(Challem et al., 1981; Nippon, 1977).*

4.5. Vitamins

Spirulina spp. has in its composition the most important vitamins, such vitamin A, vitamin B_1 (thiamine), B_2 (riboflavin), B_3 (nicotinamide), B_6 (pyridoxine), B_9 (folic acid), B_{12} (cyanocobalamin), vitamin C, vitamin D and vitamin E.

The presence of vitamin A in the microalgae comes from the conversion of β-carotene. The last one is found in large quantities in *Spirulina spp.*, 40-80% of carotenoids present in the microalgae *(Habib et al, 2008).*

4.6. Minerals

This microalga is rich in potassium, but it also contains calcium, chlorine, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc *(Habib et al, 2008).*

The iron which is in *Spirulina spp.* has a wide range of high values (550-6000 mg / kg). Through ingestion of *Spirulina spp.*, the iron is better absorbed by the body than by food chains, which could serve as a source of iron to fight anemia, for example *(Falque and Hurni, 2006).*

4.7. Photosynthetics pigments

There are some natural pigments found in *Spirulina spp.*, such as chlorophyll a, β-carotene, c-phycocyanin and allophycocyanin. These pigments are responsible for the color of the cyanobacterium *(Habib et al, 2008).*

Table 2 - Mass of the pigments in the dry *Spirulina spp.* (*Belay, 1997*)

Pigments	Mass ($kg_{pigment}/kg_{Spirulina}$)
Carotenoids	3.7×10^{-3}
Chlorophyll a	0.1
Phycocyanin	0.14

5. Reproduction

The reproduction of *Spirulina spp.* occurs by binary fraction as a form of asexual reproduction and cells division. This mode of reproduction consists of the division of a mature trichome in several pieces, from the formation of specific cells, necridial, which occurs in the analysis, giving rise to biconcave separation disks. The fragmentation of the trichome produces gliding, short chains of cells (with 2 or 4 cells), hormogonia, that moves away from the parental filament, giving rise to a new trichome. The cells in the hormogonium lose the attached portions of the necridial cells, becoming rounded at the distal extremities with little or no thickening of the walls *(Ciferri, 1983)*.

In this process, the cytoplasm appears less granulated and the cells present a pale blue-green color. Whit this process the trichomes increase in length and assume the helicoidal shape.

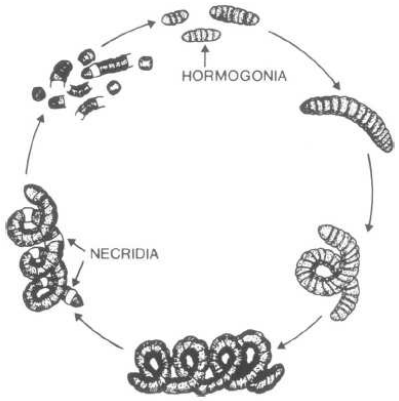


Figure 1 - Life cycle of *Spirulina spp.* (*Ciferri, 1983*)

6. Photosynthesis

6.1. Photosynthesis process

Photosynthesis is a biological process, which consists on the transformation of carbon dioxide into organic compounds, especially sugars, using the energy from the light.

This process occurs in plants, algae, and many species of bacteria. With the exception of some bacteria, like *Spirulina spp.*, using water and carbon dioxide as initial substrates, and releasing oxygen as a waste product.

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

Weak in energy Rich in energy

For *Spirulina platensis* the photosynthesis occurs in the thylakoids membranes, where the photosynthetic pigments are located: chlorophyll a, carotenoid and phycocyanin *(Cornet et al, 1998).* The process has different steps which are summarized in the well-know “Z-scheme” for photosynthesis. The explanation of this method is in Appendix I.

6.2. Photosynthesis velocity

The photosynthesis velocity depends on the rate of absorption of photons and the efficiency of conversion of these photons into chemical energy.

The quality and quantity of light energy will influence the process of photosynthesis. This means that a wavelength absorbed by a photosynthetic microorganism depends on the quantity of pigments. Regarding the quantity of light energy, it appears that the rate of absorption of photons is not proportional to the photosynthesis speed.

Through the following figure, it is possible to verify that, to different intensities, there are different stages. First there is an accelerated growth till the saturation stage, then there's an accentuated decreased after a light intensity of 300 W/m² *(Cornet et al, 1992).*

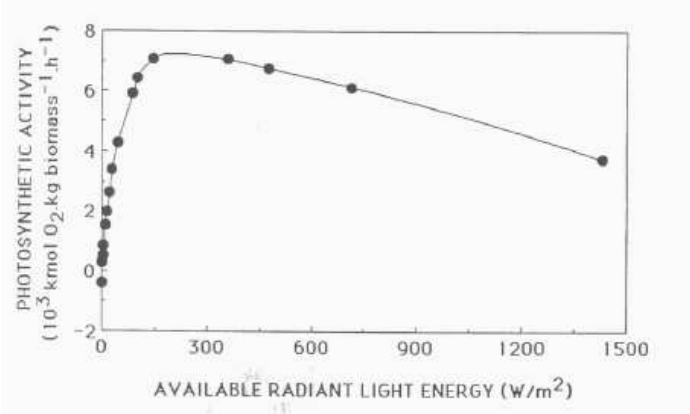


Figure 2 - Evolution of the *Spirulina spp.* culture in function of the light intensity *(Cornet et al, 1992)*

7. Culture parameters of *Spirulina platensis*

The growth of *Spirulina platensis* depends on different parameters that may affect the variation of the biomass concentration.

7.1. Physical factors

7.1.1. Light

The lighting of *Spirulina platensis* will be set according to the microalgae needs to synthesize the essential constituents for the biomass production. According to *Zarrouk (1966)*, optimum light intensity is 250 to 350 W/ m² with culture agitation. At light intensity higher than 400 W/ m², occurs the culture degeneration *(Becker and Ventakaraman, 1982).*

7.1.2. Agitation

The agitation system of *Spirulina platensis* is required to homogenize the light dispersion in order to protect the filaments from photolysis that occurs during a long light exposure. An agitation at high speed damages the *Spirulina platensis* filament and it is sensitive to high stress shear with a maximum measured between 0.06 and 0.07 Pa *(Cornet, 1992).*

7.1.3. Temperature

The optimal temperature for the growth of *Spirulina platensis* has been fixed between 35 and 37°C, by *Zarrouk (1966),* and between 30 and 35°C. The following illustration (Figure 3) shows the influence of this parameter on the biomass growth according to *Oliveira et al. (1999).* The optimum temperature is between 30 and 35°C.

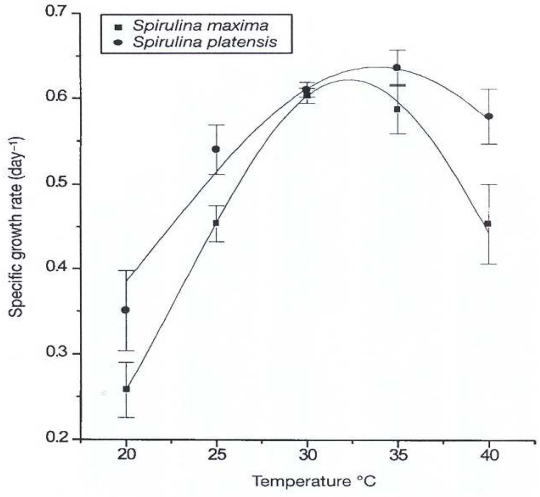


Figure 3 - Growth rate of *Spirulina maxima* and *Spirulina platensis* at different temperatures in a 4 liter reactor after, with a light intensity at 97.8 W / m² *(Oliveira et al., 1999)*

7.2. Chemical factors

7.2.1. pH

Spirulina platensis grows in alkaline conditions with a pH between 8.3 and 11 *(Zarrouk, 1966).* The following illustration (Figure 4) shows the influence of pH on the speed of oxygen production:

- The pH_{opt} is located between 8 and 10 (corresponds to the optimal value of the photosynthesis)
- The pH_{max} is located around 11.5 with a stop on the speed of oxygen production beyond this value.
- The pH_{min} is located around 6.8

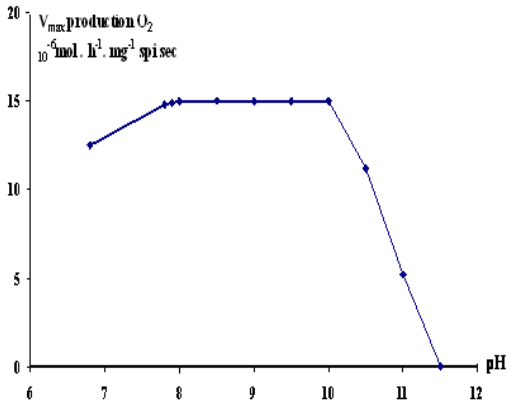


Figure 4 - pH influence on the photosynthesis speed in *Spirulina platensis*, with a light intensity of 60 klux at 40 ° C *(Zarrouk, 1966)*

7.2.2. Source of carbon

To achieve the process of photosynthesis, the carbon must be present in the culture medium composition. *Spirulina platensis* contains 47% of carbon, which is necessary to its growth. The culture medium must be very rich in bicarbonate of sodium (10.8 g/l) and sodium carbonate (7.6g/l) which are used as a source of this important element for photosynthesis *(Fox, 1999).*

7.2.3. Minerals Elements

Potassium (phosphate potassium, sulfate potassium), sodium (carbonate sodium, bicarbonate sodium, nitrate sodium and chloride sodium), phosphorus (phosphate potassium), calcium (chloride calcium), magnesium (sulfate magnesium hydrated), iron (sulfate iron hydrate) and EDTA *(Zarrouk, 1966),* are the principals minerals for the *Spirulina platensis* development. For that, any culture mediums have to contain these minerals elements.

8. Culture of *Spirulina platensis* in bioreactors

The culture of *Spirulina platensis* in the so-called not renewed bioreactors generally begins with a small concentration in biomass. The culture of this type is launched in discontinuous, until there is an exhaustion of a component of the culture medium, a change of parameters such as the temperature, pH, or a lack of light by the phenomenon of self-shading. To prevent the *Spirulina spp.* mortality because of the conditions changes, they must be maintained constant (adjust pH, temperature and adjust the middle of culture) until the concentration in biomass reaches its maximum. In this case at the end of culture the bioreactor was emptied and cleaned for a future use *(Fernandez et al., 1997).* The standard schema of the microalgae growth in not renewed medium has been established in Figure 5 *(Buchanam, 1918).*

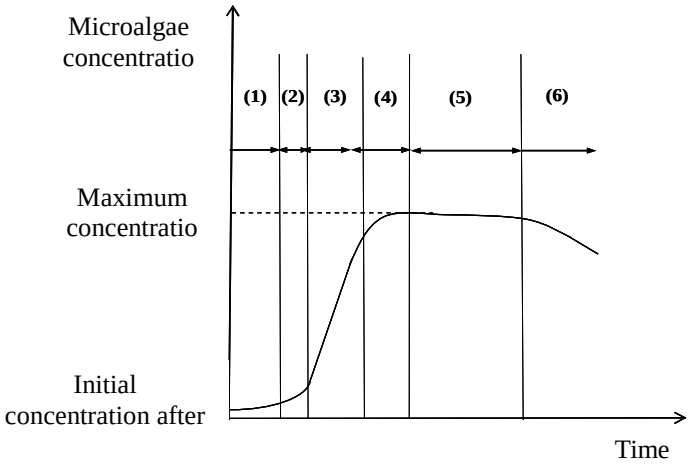


Figure 5 -Growth Curve in culture in not renewed medium *(Buchanan, 1918)*

(1): Lag phase corresponds to the adaptation of microorganisms to their environment. The duration of this phase depends, on among other things, the physiological state of cells.

(2): Acceleration phase, during which the cells begin to multiply.

(3): Log phase, during which the specific growth rate remains constant and maximum.

(4): Phase downturn, during which growth begins to be limited.

(5): Stationary phase, during which the biomass growth stabilizes.

(6): Phase generally decline due to high mortality of cells (autolysis).

During the exponential growth, the cells reproductions heat their peak. This capacity is characterized by the maximum specific growth rate, μ_{max} (s^{-1}) which intervenes in the law of evolution of the biomass concentration (C_x):

$$\frac{dC_x}{dt} = \mu_{max} . C_x \qquad \textbf{(1.1)}$$

9. Culture Medium

There are several culture mediums for the artificial cultivation of *Spirulina platensis*. Most of them are modifications of the culture medium published by Zarrouk *(Zarrouk, 1966)* based on considerations of its natural habitat and ecological: Schlosser *(Schlösser, 1982)*, Paoletti *(Paoletti et al., 1975)*, BG 11*(Vonshak et al., 1988)*, CFTRI medium (Central Food Technological Research Institut, *Venkataraman et al., 1985)*, d'Allen modified *(Vonshak et al., 1988)*, Zarrouk and Zarrouk modified.

Spirulina production in laboratory frequently uses the modified culture of Zarrouk. This medium differs from the normal Zarrouk medium, only on the carbonates and bicarbonates concentration to obtain a pH near 9.5 *(Vernerey, 2000)*.

10. Starting Culture

For new microalgae cultures, it is necessary to take into account the initial conditions, to avoid photolysis *(Fox, 1999).* In all systems of culture, the cells are on the surface and within the system and should receive the same light intensity. Therefore, the system of agitation is essential to allow a light homogenization across the culture *(Fox, 1999).*

The culture presents an optimal growth, when it is in constant motion and in the presence of a strong light intensity. This is due to the fact that all filaments often acquire brightness, and then are quickly covered by other (*Fox, 1999*).

Table 3 lists the different initial biomass concentrations used as a function of temperature and light intensity to start a *Spirulina platensis* culture. If the other parameters of pH and agitation speed are constant, the dry biomass productivity depends on light intensity and exposure of cells to light.

Table 3- Initial concentrations and light intensity used in the starting cultures

Reference	**Initial concentration ($g_{dry\ spirulina}$/l)**	**Temperature (ºC)**	**Luminous intensity (W/m^2)**	**Type of culture / reactor**	**Productivity ($g_{dry\ biomass}$/ l/ day)**
Zarrouk (*1966*)	0.05	33	50	Batch, Erlenmeyer flasks	0.500
	0.10				0.900
	0.20				1.300
	0.32				1.700
	0.50				2.000
	0.88				2.400
Cornet *et al.* (*1992*)	0.10	35	20	Batch, agitated tank	0.690
	0.15		50		0.910
	0.20		70		
Marty *et al* (*1997*)	0.10	36	47	Batch, agitated tank	0.385
	0.30		209		0.622
	1.75		304		0.812
Watanabe et Hall (*1996*)	0.20	36	296 (12h/day)	Batch, tubular reactor	0.510
Morist *et al.* (2001)	1.80	36	133	Batch, Gazosiphon photobioreactor	0.320
Travieso (2001)	0.078	Between 28 and 30		Batch, Helical tubular photobioreactor	0.400
Pelizer *et al.* (*2003*)	0.05	30	35	Batch, Mini-tanks	0.714
	0.10				0.760
	0.15				0.750
	0.20				0.670
Radmann *et al.* (2007)	0.15	30	30	Batch, Open raceway ponds	Between 0.028 and 0.046

11. Implementation and use of *Spirulina spp.*

11.1. Nutritional aspect

Spirulina spp. is known for its richness in protein, vitamins and other nutritional elements *(Peiretti et al., 2008).*

The digestion of *Spirulina spp.* is achieved due to the absence of cellulose in the cell wall, which is replaced by a layer of "fragile murein", unlike other microorganisms (yeast, *Chlorella*, ...) *(Bujard, 1970)*. This explains the ease digestion and that simply dry *Spirulina spp.* has 83 to 90% protein (casein pure: 95.1%) *(Santillan, 1974; Dillon & Phan, 1993)*.

Spirulina spp. pigments (carotenoids, phycocyanin, chlorophyll) are used as colorings the food industries, but rarely in pharmaceutical and cosmetic *(Rossi et al., 2004)*. Due to the features provided, *Spirulina spp.* can be used as supplement-food, to combat malnutrition in Africa *(Fox, 1999)* and human diet *(Ciferri, 1983);*

11.2. Therapeutic aspects

Recent studies have revealed the potential application of *Spirulina platensis* in therapy.

a) Activity antioxidant *(Cemek et al., 2008)*

b) Anti-inflammatory activity *(Remirez et al., 1999).*

c) Activity hypolipidaemic *(Devi et al., 1983; Fong et al., 2000)*

d) Anticancer activity *(Gershwin et al., 2008)*

e) Other effects of *Spirulina platensis*: several scientific studies have shown other therapeutic effects of *Spirulina platensis* as a antimutagenic (*Cevallos et al., 2008*) and an immunologic stimulant *(Subhashimi et al., 2004; Xue et al., 2005).*

Chapter II: The Photobioreactor

1. Definition of photobioreactor

A photobioreactor consists on a closed system, which in the presence of light energy, occurs biological interactions, thus allowing control over them from the culture conditions. One of the interactions that occur in this type reactor is the photosynthesis. So this may happen it is necessary the presence of CO_2 and light, in order to produce biomass from microalgae.

2. Design and general problems

The main function of the photobioreactor is the cultivation of photosynthetic microorganisms under controlled conditions, with the substrate addition necessary for its development, and the presence of light energy.

2.1. Light system

The peculiarity of a photobioreactor is to provide energy (solar or artificial) to the microalgae, to allow the completion of photosynthesis. Thus, it has to be transparent and designed so the light intensity provided is sufficient, but not lethal to the development of culture.

As already noted the growth of microalgae, as well as the biomass production, depends on the amount of light received by the microalgae. There are two key factors to take into account in the photobioreactor design *(Olivo, 2007)*:

a) Quantity and quality of light provided by the device (intensity, the emission spectrum and the number of lamps in the device);

b) Quantity and quality of light really available to the microalgae in the system center, since the luminous flux incident is different from that that crosses the reactor walls and the culture;

The really available luminous radiation for the culture depends on cell concentration.

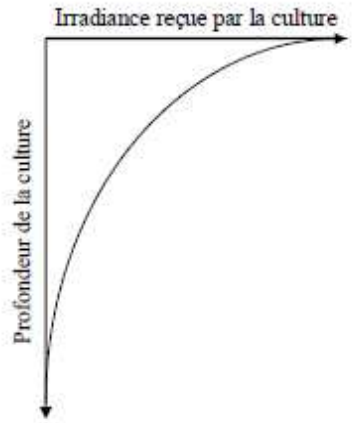


Figure 6- Schematic representation of the attenuation of light depending on the thickness of culture *(Olivo, 2007)*

This phenomenon is called self-shadow (absorption and diffusion of light by microalgae), and the scarcity of light makes the distribution of light energy a heterogeneous system.

The uneven distribution of light, depending on the thickness of the culture, leads to the development of microalgae located:

a) On the surface, due to a higher probability of light intensity incidence is very strong, which causes the appearance of an photoinibition area;

b) In depth, the effects of self-shadow can lead to photoalimentation due to scarcity of energy, or even a break in the respiratory mechanism.

Thus, the light access should have an important weight in the photobioreactor design.

2.2. Agitation system

The agitation and scouring systems are used in order to promote the culture homogenization, (allowing the development of various reactions in the photobioreactor), the access of light and the liquid-liquid and gas-liquid transfers, the limit areas of photolimitation and photoinibition and the prevention of the biofilm formation. However, these systems may weaken the biological material.

The hydrodynamics of the agitation system is a stressful factor for cell growth, since it can cause disturbances in the cell physiological state as well as in its growth *(Sanchez et al., 1999).*

This is due to the strong shear at level of the gas injector, training of cells by flotation and scattering as bubble in the surface *(Sanchez et al., 1999; Barbosa, 2003).*

Thus, the agitation / scouring system for the photobioreactor must allow not only the system homogeneity, but also preserve cell integrity.

3. Photobioreactor technology

There are alternative technologies to optimize the photobioreactors productivity and its applications on the reactor geometry.

Based on the literature it was possible to verify that there are different types of photobioreactors (Appendix II), but the two types of geometry more current are: the photobioreactors plats (flat geometry, used preferably in industrial production) and photobioreactors tubular (cylindrical geometry, laboratory use). For cylindrical photobioreactors, there are various possible configurations *(Tredici, 2004)*:

- a large tube and forming a vertical column;
- two tubes of different diameters arranged one inside the other forming an annular chamber;
- a tube placed in the soil and diameter moderate but significant length arranged in a serpentine;

- a tube of small diameter and large length wound helically around a turn;
- several small diameter tubes arranged in parallel and vertical.

The cylindrical photobioreactors are widely used because their design is simple and easy to scale for large volumes of several hundred liters *(Tsygankov, 2001).*

Based on the literature, it is also possible to verify the most favorable geometry and conditions necessary to obtain an efficient productivity.

Table 4 - Productivity obtained by different types of photobioreactor

Type of Photobioreactor	**Productivity, $g_{dry\ Spirulina}$/ l/day**
Cone-shaped helical tubular photobioreactor *(Watanabe & Hall, 1996)*	0.51
Spiral form of a tubular photobioreactor *(Carlozzi and Torzillo, 1996)*	0.86 (laminar regime) 1.06 (turbulent regime)
Vertical column photobioreactor *(Marty, 1997)*	With 47 W/m²: 0.31 With 118 W/m²: 0.46 With 209 W/m²: 0.64 With 304 W/m²: 0.79
Gazosiphon photobioreactor with internal loop *(Vernerey, 2000)*	0.9 to1.0
Gazosiphon photobioreactor *(Morist et al, 2001)*	0.32
Helical tubular photobioreactor *(Travieso, 2001)*	0.40
Mini-tanks *(Pelizer et al, 2003)*	0.67 to 0.76
Open raceway ponds *(Radmann et al, 2007)*	0.028 to 0.046

Chapiter III: Materials and Methods

III.1 Photobioreactor design for familiar production of *Spirulina platensis*

1. Introduction

As already mentioned previously, *Spirulina* is commercially available, being produced through a commercial process, plant-scale cultivation, leaving as final product under the form of tablets, capsules, powders, drinks, etc… The application of this method not only requires considerable investments, which results in high price of the product, but also leads to the loss of some nutrients from *Spirulina*, after drying process. Sometimes, due to drying process, some ingredients were deteriorated, resulting in a terrible flavor *(Li et al., 2004),* whereas in fresh product it does not happen.

As an alternative to the problem, it was proposed a reactor design to allow the cultivation and consumption of fresh *Spirulina* at home, avoiding the damage to public health caused by the counterfeiting of food *(Li et al., 2004).* This need has already been taken into account by *Li et al. (2004),* but the reactor was not implemented on a commercial level. However, in industrial and laboratory level the implementation of a reactor for the *Spirulina platensis* production was well suited.

2. Objective of the photobioreactor conception

The design of this reactor would enable the consumer to cultivate and consume fresh *Spirulina spp.*, at home, and it would change high-grade nourishing health foods into daily foods that every one and every family could afford *(Li et al., 2004).*

With a analysis of *Spirulina platensis* cost, it was possible to verify that the price of dry *Spirulina platensis* is very high at the moment, for instance one bottle of 100 g with 100% of *Spirulina* dehydrated in flake form cost 14€ (*www.algosud.com)* and one bottle of 135g with 100% of natural *Spirulina platensis* in powder form, cost 26,10€ (*www.aromatic-provence.com).*

3. Methods

3.1 Need Analysis

The need analysis is the process of identifying and evaluating needs in a community. So, this method takes place in two steps: finding and validation of the need (*www.techniques-ingenieur.fr*).

3.1.1 Need Identification

This step allows expressing the purpose and limits of the product in study. Therefore, it is necessary to clarify the fundamental requirement that justifies the conception of this product. Thus, to find the need, it is necessary to put three fundamental questions about the product design (*www.techniques-ingenieur.fr*): Who (or what) does the product serve?; Who (or what) is it for?; For what purpose?

3.1.2 Need Validation

After the need identification that the product should accomplish, it is necessary to check its stability answering the following three questions (*www.techniques-ingenieur.fr*):

- Why does this need exist?;
- What could make it disappear? The changing?;
- Risk of losing the need? The progress?.

3.2 Functional Analysis

The functional analysis is the fundamental step of the product analyses, for the product creation or improvement. This step consists in finding, characterize, prioritize and/or enhance the functions of the product expected by the user. The functional analysis is performed in several steps:

3.2.1 Profile life system

After the collection of information, through the literature research carried out, this phase allows establishing and identifying the life expectancy of the product, as well as the stages of the reactor life cycle.

3.2.2 Function identification

The identification of the functions is obtained through the relationship established between the new product and the environment to which it will be exposed. There are several types of methods which may be used to perform this analysis, such as the intuitive method, the dissatisfaction, competition, APTE, SADT, FAST, GRAFCET, and SAFE.

3.2.3 Functions characterization and quantification

Once the functions are identified, it is necessary, firstly, to order the functions in a logical sequence, followed by criteria definition for each function, which will enable us to carry out the

choice of a solution: the characterization of functions. For that, the Functional Specifications Document (FSD) was used, a document that allows to express the need in terms of functions that in the future the product will accomplish (*www.techniques-ingenieur.fr*).

Thus, to obtain the FSD, it is necessary to carry out:

a. Function identification;

b. Criteria expression: each function should present one or more criteria, in order to verify the importance of the function;

c. Define a level for each criterion: the level of a criterion is a size identified in the scale adopted for the function criterion;

d. Flexibility: consists in defining if the levels of criteria should be respected or may be negotiated. The levels of flexibility can be classified in: **F0**: Imperative level (no flexibility); **F1**: Level slightly negotiable; **F2**: Negotiable level; **F3**: Very negotiable level.

3.2.4 Functions hierarchy

This phase allows quantifying the relative importance of the functions. To perform this, three steps have been used (*www.techniques-ingenieur.fr*):

a. Comparison between the functions

The objective of this phase is to compare the functions one by one by, with a note attribution, using a matrix. The scale selected for this classification is: **0**: level equal; **1**: level slightly higher; **2**: medium level of superiority; **3**: high level of superiority

b. Estimate the weight of each function

After classifying the functions, the sum of points of each function to determine the weight percentage of each function was carry out.

3.3 Solution research

For the photobioreactor development, it is necessary to do a detailed literature research, for the design development of a new photobioreactor or to improve an existing one.

This requires creativity in order to associate all the information collected in ideas, in order to conceive a new photobioreactor or to solve a problem of an already existing photobioreactor. Therefore, it is necessary to have some considerations about main aspects of a photobioreactor, as the position of the lighting system, the agitation system, the harvest system, the photobioreactor geometry and the cleaning system.

In this step, many options have been exposed, but some of the options can not be implemented

in the photobioreactor.

3.4 Solutions select

This step aims at reducing the number of solutions to obtain a better perception of the final product. For that, it was turned back to the method of functions appreciation.

This method allows quantifying the solutions to verify the execution of the different functions, using a matrix between the solutions and the functions identified.

The applicability of the method is carried out in three steps:

a. Solution Quantification

This stage allows to attribute a note to the analyze solution, based on the fulfillment of the function to which the comparison is provided.

The scale attributed for this classification, has a range between 0 and 4, which represent: **0**: not fulfilling the function; **1**: insufficient compliance; **2**: mediocre compliance; **3**: compliance above average; **4**: excellent compliance

b. Note of appreciation of the solution

After the solution classification for the different functions and using the results, in percentage, of each function, through Functions Hierarchy method, it was determinate the appreciation from the solution using the following equation:

$$Appreciation\ note = \sum_{i=1}^{n} \frac{(solution\ classification) \times (\%\ function\ classification)}{100} \quad \textbf{(1.2)}$$

c. Reduction of the solution number

By the analysis of the results, it was proceed to the elimination of the results that have a low note of appreciation. In the case this method is not well succeeded, ie if the number of eliminated solutions is low, it is needed to verify which solutions, have lower classifications in the key functions so as to be eliminated.

III.2 Follow-up of *Spirulina platensis* culture in the photobioreactor

1. Introduction

The culture of *Spirulina platensis*, in a bioreactor keeps the luminosity and the temperature constant, during the entire period of culture, and also a non-destructive restlessness of filaments. The growth of these microalgae depends on temperature, light, pH, culture agitation and the composition of the culture medium *(Cornet et al., 1998).*

2. Material

2.1. The strain of Spirulina platensis

The origin of *Spirulina platensis* used in this work comes from a culture from the south of France (MR. Jourdan to Mialet and Mr. Nogier St Paul de Caisson).

2.2. Photobioreactor

The culture of *Spirulina platensis* has been carried out in a photobioreactor at the laboratory of LAGEP - University of Lyon 1 (Figure 10).

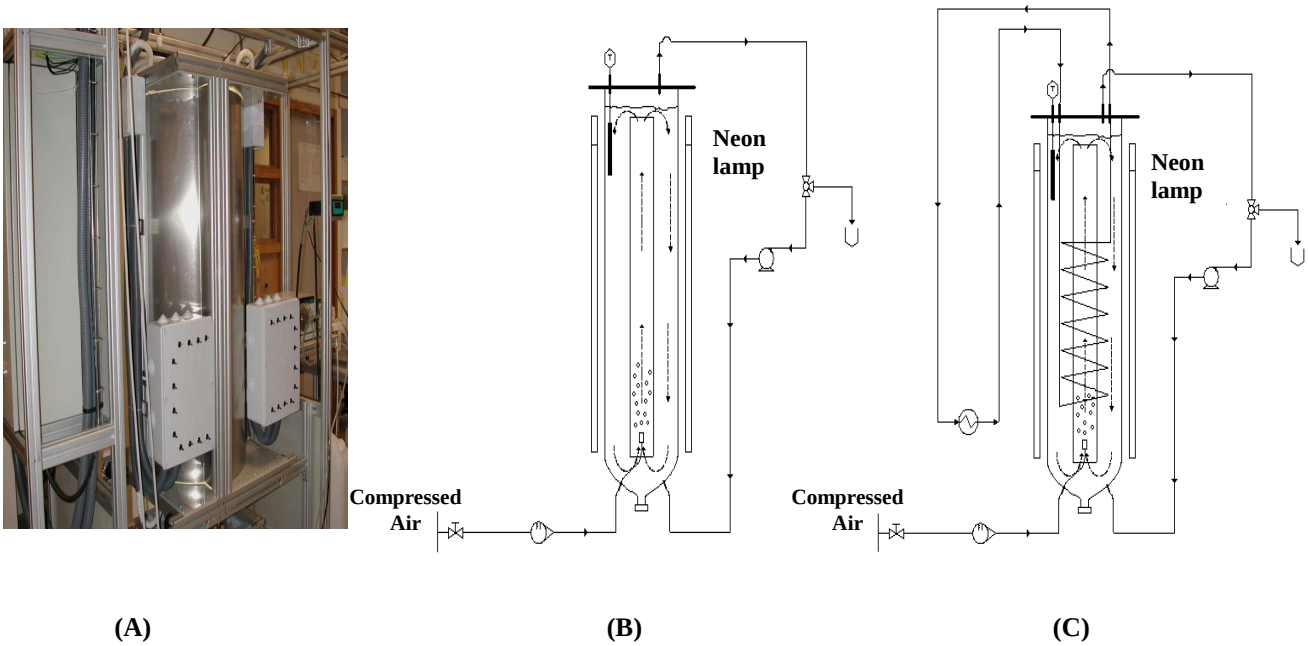


Figure 7 - (A): General view of the photo-bioreactor "vertical cylinder" of *Spirulina platensis* in LAGEP; **(B):** Diagram of the LAGEP photobioreactor used in some experiments, without temperature regulation; **(C):** Diagram of the LAGEP photobioreactor used in some experiments, with a system of temperature regulation

The photobioreactor is composed of several parts (Appendix V):

- Glass tank of 40 l of volume, a internal diameter 0.22 m, thickness 0.015 m, length 1.27 m;
- Agitation system and aeration system is done by a bubbling system: a central PVC tube, with a length of 1.10 m and an internal diameter of 0.94 m, placed inside the reactor;
- A lighting system mounted on a lid of aluminum: 20 fluorescent lamps (15 neon lamps and 5 sunlamps) providing a total light intensity of 100W / m^2;
- A plastic top cover to ensure the passage of a thermometer;
- A peristaltic pump (Cole Pramer Instrument co., Chicago) as a sampling system by pumping;
- For some experiments, a temperature regulation system (Cryo-thermostats compacts RCS 6, Lauda, Germany) was used, through a coil, in stainless steel of 85 cm high, with internal and external diameter of 14 cm and 16 cm, respectively, coupled to a thermostat.

2.3. Culture medium

The culture medium used is Zarrouk modified (Zarrouk, 1966), it contains different concentrations of: $NaHCO_3$ (AnalaR NORMAPUB), $NaNO_3$ (Prolabo), K_2HPO_4 (Prolabo), K_2SO_4 (Riedel de Haeu), NaCl (Baleine, de mer fluoré), $MgSO_4$.$7H_2O$ (ACROS OrganicSsel), $CaCl_2$ (Prolabo organics), $FeSO_4$ $7H_2O$, EDTA, Na_2CO_3 (Carl Roth) and tap water.

2.4. Other chemical products

In the culture followed, sometimes other products are used, like: sodium hypochlorhydria, H_2SO_4 at 95-97% (Sigma-Aldrich) at 0.5 M and HCl at 95-97% (Sigma-Aldrich) at 0.2 M.

3. Methods

3.1. Starting cultures

To obtain a culture without contamination (such as bacteria, viruses and protozoa) it is necessary a chemical sterilization of the photobioreactor.

This procedure consists in filling the photobioreactor with sodium hypochlorhydria at 1%. After 15 minutes the photobioreactor was emptied and then rinsed 2 times with tap water *(Tredici 1998).*

To make the preparation of a culture, it is necessary to fill, first the photobioreactor with the culture medium. In order to obtain the desired biomass concentration, it is necessary to add the inoculation of culture into the photobioreactor. Then, the systems for lighting, temperature and agitation, are set to conditions required for each of the performed experiments.

3.2. Preparation of culture medium

The culture medium was prepared in a 2000 ml Erlenmeyer flask for 37 liters of culture medium. This volume was diluted in the bioreactor with tap water, with 3 liters of inoculation, to obtain the initial intended concentration. The initial concentration of each inoculation is obtained using the dry biomass weight method.

The quantities needed to prepare 37 liters of culture medium are listed in table 8.

Table 5 - Composition of culture medium (*Cornet et al, 1992*)

Components	Concentration (g/l)	Quantity of product (g) for 37 liters
$NaHCO_3$	10.800	378.00
$NaNO_3$	2.500	87.50
K_2HPO_4	0.500	17.50
K_2SO_4	1.000	35.00
NaCl	1.000	35.00
$MgSO_4.7H_2O$	0.200	7.00
$CaCl_2$	0.040	1.40
$FeSO_4.7H_2O$	0.010	0.35
EDTA	0.008	0.28
Na_2CO_3	7.600	266.00

3.3. Flow agitation

The culture agitation, for this airlift photobioreactor, is ensured by an air circulation bottom-up in the center of the tube and top-down along the external walls. The air flow is controlled by a rotameter (Emerson Electric SA., France) at 25 l_{air}/h, and it is composed by 0.03% CO_2. This CO_2 composition will enhance the development of this microalga.

3.4. pH regulation

For each experiment, the pH of the culture in the photobioreactor was measured using a pH meter (pH Cheker, HANNA, France). If it was high (> 10.5), a solution of sulfuric acid 0.5 M was added directly in the culture (absence of automatic system to adjust the pH in the bioreactor), at the top of the photobioreactor. After the acid addition, it was used a peristaltic pump (Corl Parmer Instrument co. Chicago) in a closed loop during thirty minutes.

3.5. Sampling system

For the accomplishment of the different methods it is necessary to collect a representative volume of the 40 liters bioreactor. A flow of *Spirulina platensis* was conducted for thirty minutes with a peristaltic pump (Corl Parmer Instrument, Chicago) in a closed loop before each sample of 200 ml.

3.6. Parameters measured during follow-up of the culture

The determination of biomass concentration is obtained by the accomplishment of two methods, in order to verify if there is a large discrepancy between them.

3.6.1. Determination of dry biomass weight

This method involves taking 50 ml of *Spirulina platensis* culture from the bioreactor. This volume has been filtered through a 40µm filter, and rinsed successively with 100 ml NaCl 0.9 g / l, to allow the minerals separation of the culture medium, and to avoid the broken of the cells. Posteriorly was used 100 ml of distilled water for a total recuperation of the sample. The obtained cake was dried in an oven at 110 ° C to constant mass.

3.6.2. Determination of biomass concentration using Optical Density (OD)

To achieve the measurement of optical density, it is necessary to make a calibration curve with a wavelength of 750 nm, using a spectrophotometer UV/VIS (Cary 50 Probe, Varian). This curve is necessary for each of the performed experiment.

To obtain the curve, it is necessary to remove 50 ml of concentrated culture in the photobioreactor, and subsequently 25 ml was removed from the 50 ml to make 5 dilutions with the culture medium, using volumetric flasks of 10 ml each. The dilution performed for 5 volumetric flasks is made to obtain an absorbance below 1.0. It should be noted that this curve is obtained with the dilutions in a single day.

The measure of optical density for *Spirulina platensis* culture is proceeding with the same wavelength used in the calibration curve, 750 nm.

The measurement was performed at this wavelength, since the exopolysaccharides do not absorb, presenting only the principal *Spirulina plantensis* pigment's of photosynthesis.

3.6.3. Salinity

For this method a densimeter was used in the obtained filtrate after the culture sample filtration. The reading is proceeding at the meniscus level obtained, according to *Jourdan* (1999), the density (DT). The determination of the density at 20°C (D_{20}) is necessary to know the temperature of the

filtered (° C).

$$D_{20} = DT + 3.25 \times 10^{-4} \times (T - 20) \quad \textbf{(1.3)}$$

The salinity and D_{20} are related by the following equations:

If $D_{20} > 1.0076$:

$$Salinity\ (g/l) = SAL = 1250 \times (D_{20} - 1.0076) + 10 \quad \textbf{(1.4)}$$

Otherwise:

$$Salinity\ (g/l) = SAL = 1041 \times (D_{20} - 0.998) \quad \textbf{(1.5)}$$

3.6.4. Total inorganic carbon concentration

For the accomplishment of this method a titrimetric dosage was used *(Degremont, 1978)*. This method consists in performing two consecutive titrations, using a sample of filtrate, 20 ml, with the addition of hydrochloric acid of 0.2 M.

The first titration consists on the acid addition in the presence of an indicator, phenolphthalein, to the point of turning the indicator. This is due to the transformation of carbonate ions CO_3^{2-}, present in the sample, in bicarbonates ions HCO_3^-.

For the second titration it is used the indicator bromophenol blue, to promote the conversion of HCO_3^- ions in carbonic acid H_2CO_3, with the change of the blue color indicator to yellow.

Thus, through the concentration of these ions, the concentration of total inorganic carbon (C_{inorg}) is determined, given by the following equation:

$$C_{inorg} = C_{HCO_3^-} + C_{CO_3^{2-}} = \frac{V_1 . C_{HCl}}{V_{sample}} + \frac{V_2 . C_{HCl}}{V_{sample}} \quad \textbf{(1.6)}$$

Where: V_1, V_2 - volume added of HCl (ml)

3.6.5. Productivity and growth rate

The productivity of *Spirulina platensis* biomass is given by the following formula *(Danesi et al., 2004):*

$$Produtlvity(P) = \frac{C_{x,final} - C_{x,0}}{\Delta t} \quad \textbf{(1.7)}$$

Where: $C_{x,\,final}$: Final concentration or maximum biomass

$C_{x,\,0}$: Initial concentration

Δt: Duration culture.

The growth rate (μ_{max}) is obtained using the equation **(1.1)**.

3.6.6. Determination of the concentration of phycocyanin (C-PC)

The blue pigment concentration of *Spirulina platensis* is given by the following equation *(Silveira et al., 2007):*

$$C - PC\ (mg/ml) = \frac{A_{620} - 0.474 \times (A_{652})}{5.34} \qquad \textbf{(1.8)}$$

Where: A_{620}: Maximum absorbance of phycocyanin at 620 nm.

A_{652}: Maximum absorbance of proteins present in *Spirulina platensis* at 652 nm.

3.7. Experiences performed

Different experiences were performed to verify the influence of temperature on the culture evolution. The second experiment was carried out not only to verify the previous objective, but also to simulate a family production. The following Table exposes all the experiences performed in the photobioreactor and the conditions of each one of it.

Table 6 - Parameters condition for each experiment

Parameters	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
pH	9,8 – 10,53	9,52 – 10,76	-	10,12 – 10,46	10,15 – 10,66
Temperature (°C)	34 - 37	34 – 37	35	33°C during 48 h; 35°C	35°C
Light source	8 lamps	8 lamps	20 lamps	6 lamps	6→12→ 20 lamps
Light intensity (W/m^2)	40	40	100	30	30 →60→100
Temperature Regulation	N° lamps+ thermometer	N° lamps+ thermometer	Thermostat + thermometer	Thermostat + thermometer	Thermostat + thermometer

Chapter IV: Results and Discussion

IV.1 Photobioreactor design for familiar production of *Spirulina platensis*

The design of a new photobioreactor for *Spirulina platensis* can lead to a better creative research regarding new ideas and applications, as well business initiatives for the future. Some details of the design process are presented in the next sections.

1. Need Analysis

1.1. Need Identification

The photobioreactor answers to a consumer need concerning *Spirulina platensis*, as described in the represented diagram (Figure 7).

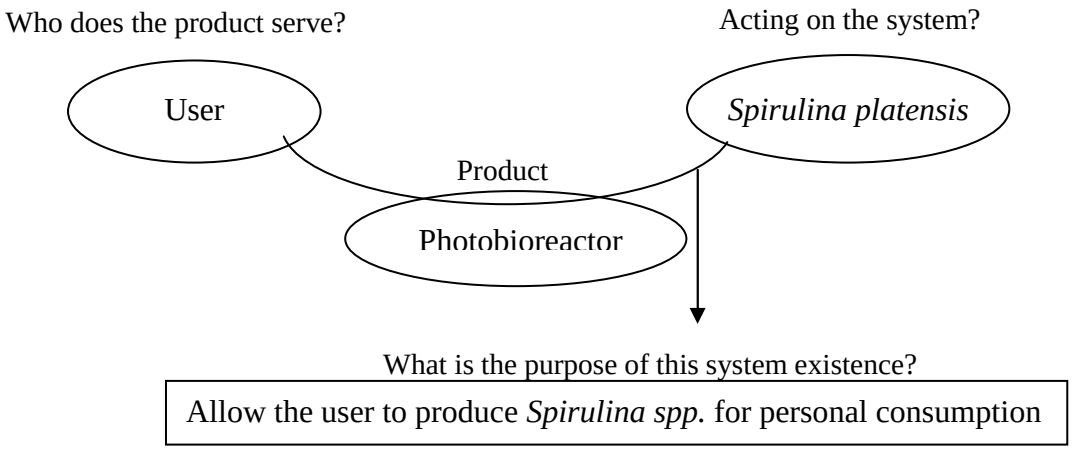


Figure 8- "Horned beast" diagram

Based on the identified need, the reactor must be conceived in a way that the reactor cost is not very high, among 30 to 60 €. According to the literature *(Falquet and Hurni, 2006)*, a user can consume per day about 1 to 1.8 g / l of *Spirulina spp.*. A key aspect to take into account in the reactor design is the capacity that means the occupation area on the ground and its weight. The medium productivity is around 0.3 g/l/day. The culture has not to be much diluted. Then to get a correct weight to be displaced from outside to inside, the capacity was limited to 10 liters.

1.2. Need Validation

a) Why does this need exist?

This project intends to changes the situations in which *Spirulina platensis* is produced (in

plants), as well as change its sole destination to stores and hospitals. Thus, the invention does not only enable the nutrients of *Spirulina spp.* to be stored and utilized more completely, but also develops and popularizes more quickly the edible *Spirulina spp.* to meet the needs of the public for health foods. As some people have expressed that they are looking for a way to find cheaper *Spirulina spp.* for their own consumption.

b) What could make it disappear? The changing?

The invention can fall in forgetfulness due to poor adherence of the product by the user. If there is a strong adherence, this can lead to consumption of other algae with properties similar to *Spirulina spp.*. The *Spirulina spp.* produced in some parts of the World (China, Africa) can be several times (10 times) cheaper. When *Spirulina spp.* is to be produced in the acceptable qualities on the northern country markets, the need might disappear.

c) Risk of losing need? The progress?

The need of *Spirulina spp.* consumption by the user will not disappear. But the cultivation of this will, since it may become a great risk once the user may prefer to consume industrialized *Spirulina spp.*. Nevertheless, the need may change depending on the needs and accession by the user, as well as attract other application areas (hospitals, cosmetic, etc. ...).

2. Functional Analysis

2.1. The profiling system life

The life time of this photobioreactor was limited to 6 years with a daily maintenance, and to 3 years without it. This can be verified through the degradation of the materials used in this invention; the culture of *Spirulina spp.* becomes less endowed in nutritional power, and favours the invention commercialization.

2.2. Functions identification

For this phase it was turned back to the APTE method. This consists in connecting the external elements to the product.

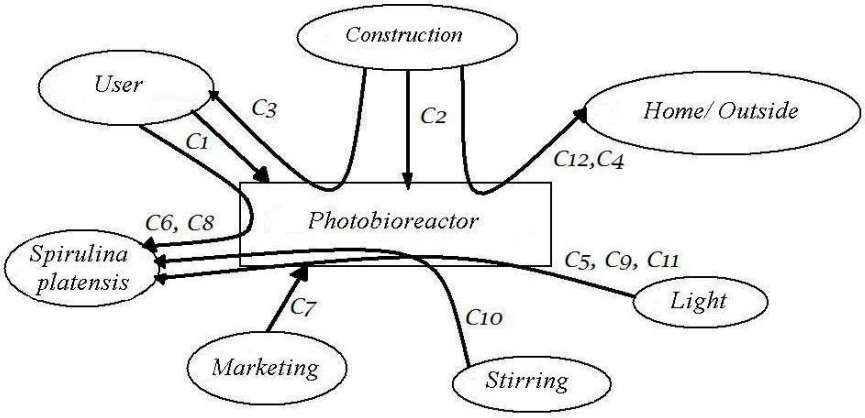


Figure 9 -The octopus

The functions obtained by this method:

C_1 : Allow the user to maintain the photobioreactor

C_2 : Allow the storage, mounting and unmounting of the photobioreactor

C_3 : Ensure the safety of the user

C_4 : Allow the photobioreactor to be moved from the outside to the inside, or vice-versa

C_5 : Bring light power regardless of the ambience

C_6 : Allow the *Spirulina platensis* to be harvested

C_7 : Allow to be known by the potential users

C_8 : Allow to produce *Spirulina platensis* to be consumed by the user

C_9 : Ensure adequate temperature to the *Spirulina platensis* culture medium

C_{10} : Allow to homogenize the culture

C_{11} : Ensure a yield maximum luminosity

C_{12} : Resist to the environment

2.3. Functions characterization and quatification

To obtain the characterization of functions and their classification, the FSD method was used, and thus obtaining the Table 6 and 7.

2.4. Functions Hierarchy

The result obtained by the implementation of this method is shown in the table below.

Table 7- Results obtained from the functions hierarchy

Functions	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
Percentage/ %	6.04	5.49	8.24	18.1	5.49	2.75	3.30	13.2	9.89	13.2	13.7	0.549

This table shows that the functions F_4 (Ensure the safety of the user), F_8 (Bring light power regardless of the ambience), F_{10} (Ensure adequate temperature to the *Spirulina platensis* culture medium) and F_{11} (Allow to homogenise the culture medium), have the higher percentage, making these functions the most important ones in the reactor design.

3. Solutions

After the exposure of many proposed solutions, some of them have been eliminated. The criteria of the functions allow the solutions to be changed and adapted. The numbers of solutions obtained are illustrated in the Appendix III.

4. Selection of solutions

The quantification of solutions was made to verify the execution of the functions in the several solutions, and the results of this analysis show that the solutions S_2, S_3, S_4 and S_5 placed in the Appendix IV, are the solutions that best accomplish all the proposed functions. The selection of those solutions was made, taking into account the light position, the geometry that easily allows the arrangement of the reactor, and the agitations system for homogenization. The selected solutions will be analyzed, later on, with more detail.

5. Basic technical parameters for the reactor development

5.1. Agitation

The choice of an agitation system is conditioned by the culture volume and the geometry of photobioreactor, as can be visualized on the graphs presented.

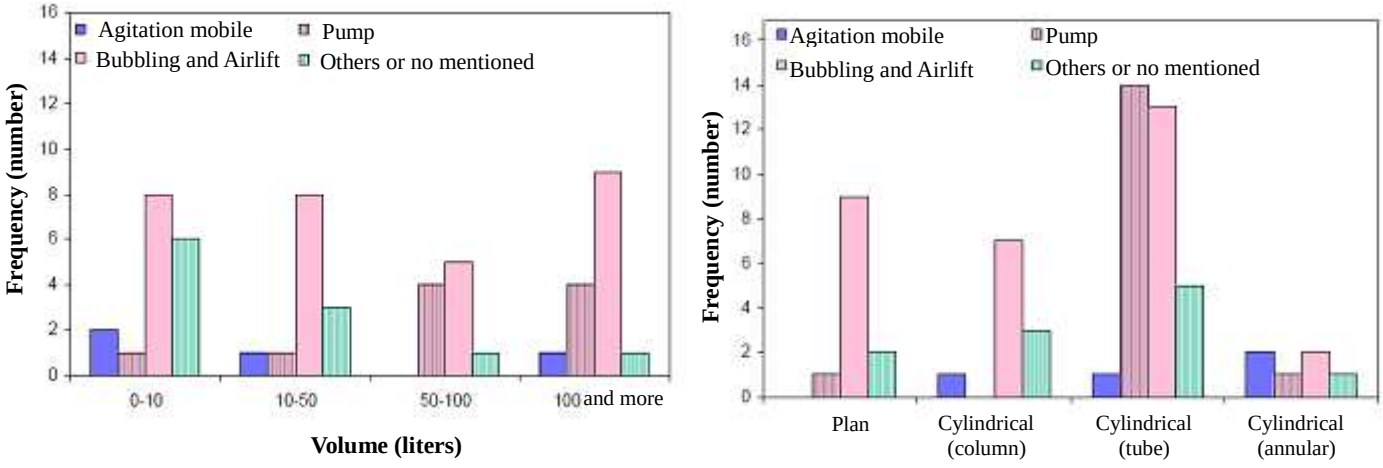


Figure 10- The different stirring used in each volume capacity (A) and geometry (B) of the photobioreactor *(Olivo, 2007)*

Therefore, since the volume set to the photobioreactor is 10 liters, the more advisable system is the airlift or the system of simple bubbling, regardless of the geometry chosen for the photobioreactor. This system of agitation is the most basic and cheapest, and it consists in the injection of gas located in an area of the reactor, causing the natural movement of the culture, because of the difference in weight of water between airy zones and not airy.

It is also necessary to refer that this system is not only used for the culture agitation, but also for cleaning the reactor walls, depending on the position of light chosen for the photobioreactor. To help this injection it is required an external device, a pump.

5.2. Luminosity

There are different forms to improve the access to light in the microalgae and to standardize the lighting over the surface.

The system brings the light energy in photobioreactor which will solve some of the issues, in one hand, geometry and volume of the enclosure, and, on the other, the processes and techniques used for the agitation and the circulation of the culture.

The geometry of the photobioreactor system should as far as possible favour a high ratio of the illuminated surface on the culture volume, while reducing the congestion and maintaining in line with the desired objectives of biomass concentration and physiological needs of the cultivated microorganisms.

The processes and techniques of agitation and the circulation of microorganisms within the culture must promote maximum access to every algae cell to adequate irradiance (maximum alternating between light and dark areas) and minimize the formation of deposits on the reactor walls (biofilm).

To reduce the level of the electrical system, it was proposed the possibility of the photobioreactor to capture the solar rays when it is outside. This will only be possible through appropriate inclination, for instance, for France it would be 45 °.

For the lighting system, it is intended that the lamps of the system are always available to the user, and that they emit light in the visible range. Thus the selected lamps for the proposed solutions will be of fluorescents tubes or halogens type. The photobioreactor must also have solar lamps so that it promotes the culture development under normal conditions.

6. Conclusion

The reactor conception was divided in five stages: the need identification, the functional analysis, the bibliographical research, elimination and, finally, the selection of solutions.

The technological parameters analyzed, for the different solutions selected, are: the type of agitation to implement, as well as the illumination used, because they have a great influence on the development of the culture.

The reactor’s design is based on the criteria presented in the FSD.

Table 8 - FSD in table form

Number	Functions	Criteria	Levels	Flex.
F_1	Allow the user to maintain the photobioreactor	Cleaning	- Manual or Automatic	F2
		Supervise electrical equipment	- When a lamp must be changed (maximal 1 month)	F1
		Quality culture	- Bacteriological analysis, every 6 months	F0
		Culture medium change	- One time every 40 or 60 days	F0
		Area on the ground	- Can be placed on a cabinet	F2
F_2	Allow the harvested *Spirulina platensis*	Adapted way	- Filtration (pores 20 to 10 µm)	F0
		Frequency	- 1 time / day	F0
		Duration	- 10 ± 5 min	F2
		Quantity	- <½ total of volume	F0
F_3	Allow to produced *Spirulina* to be consummed	Culture medium		
		- Quality	- No pathogens	F0
		- Storage	- 3 days at most at 4°C	F1
		- Quantity	- The same as the harvest	F0
		Quality	- No pathogens	F0
		Quantity	- minimal: 1 g / day /person	F1
F_4	Ensure the safety of the user	Electricity		
		- Safety Distance	- 1.5 m	F0
		- Voltage	- 12 V at 220 / 240 V	F0
		- Electrical Wires	- Waterproof	F0
		- Circuit breaker	- Anomaly in the equipment	F0
		Instrutions	- Detailed information and pictures	F3
		Security	- Equipment necessary	F1
F_5	Resist to the environment	Materials		
		- External conditions	- sun, rain, wind	F1
		- Internal conditions	- shock of the reactor on the boden	F1
F_6	Allow the storage, mounting and unmounting by the user	Difficulty	- Easy / medium, by a person without specific knowledge, but not a child	F3
		Durations	- <30 min – 1h de first time	F2
		Conditions	- Storage without humidity	F1

Table 9- FSD in table form (continuation)

Number	Functions	Criteria	Levels	Flex.
F_7	Allow the photobioreactor to be move from the outside to the inside, or vice-versa	Position Quality System Stability Volume Weight	- Still vertical - Intact, no scratches - Balance of reacteur - 10 litros - < 15 kg	F1 F0 F0 F1 F2
F_8	Bring light power regardless of the ambience	Light intensity necessary Luminosity Lamps - Type Electrical mode Duration	- 80 to 200 W/m² - Avoid glare - Solar, LED, Fluorescent, Neon, Incandescent, Halogen - Manual - Automatic - from 12 to 24/24 h	F0 F1 F1 F2 F2 F2
F_9	Ensure a yield maximum luminosity	Selections - spectrum Application - geometry of the reactor - position - material - thickness	 - 400-700 nm - Rectangular, Cylindrical - Central, Top, Sides - Compatibilty with pH 11 and light transmission - 0.4 to 0.5 cm	 F0 F2 F2 F1 F1
F_{10}	Ensure adequate temperature to the *Spirulina platrensis* culture medium	Light -Temperature	 - 30-37°C	 F1
F_{11}	Allow to homogenize the culture	Stirring	- Agitator or air bubbling	F2
F_{12}	Allow to be known by the potential users	Marketing	- Television, newspaper, web	F3

IV.2 Follow-up of *Spirulina platensis* culture in the photobioreactor

1. Light intensity in the photobioreactor

The light intensity is a major factor that contributes to the growth of *Spirulina platensis*, as all of the microalgae that accomplish the photosynthesis. Therefore, the measurement of light intensity emitted by the photobioreactor lighting system was carried out, using a luxmeter, with a range of 0-20000 lux, positioned at a constant height (luxmeter and bottom of the reactor) and distance (luxmeter and lamps) of 0.64 m and 0.21 m, respectively, when the photobioreactor was empty. The measurements were accomplished for 5, 8 and 15 lamps (30 W each), with and without solar lamps.

The results of these measurements are represented in the following figure.

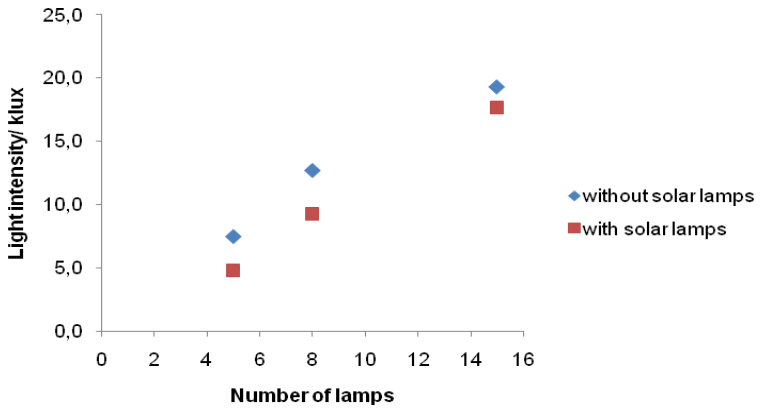
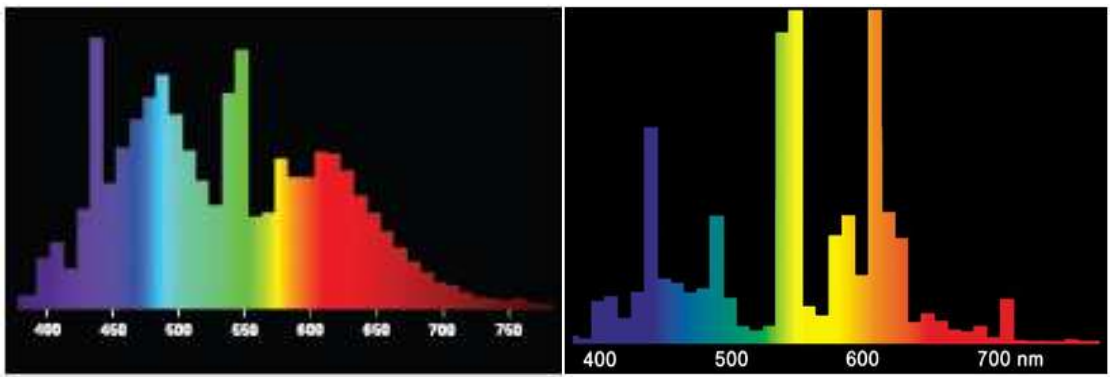


Figure 11 -Representation of the measurements results for the light intensity in function of the number of lamps, with a constant height and distance between the lamps and luxmeter of 0.64 m and 0.21 m, respectively.

The solar lamps are chosen to simulate the light radiation from the Sun, as can be seen in the emission spectrum of lamps (Figure 12.A). The neon lights present in the light system of this photobioreactor have been selected because of the study of the blue pigment, phycocyanin, growth in the *Spirulina spp.*, since this pigment absorbs a wavelength of 620 nm (Figure 12.B).



(A) **(B)**

Figure 12 -Emission spectrum of solar lamps (A) and neon lamps (B) (www.osram.fr/osram_fr/).

2. Calibration curves of OD – *Spirulina platensis* concentration

The calibration curve is given by different dilutions of a culture sample. In each experiment the calibration curve has been done when the biomass concentration of *Spirulina platensis* is high enough for the calibration curve included all the OD results obtained. Therefore, the calibration curve was performed after each experience (Appendix VI).

3. Cultures with constant light intensity

3.1. Experiment 1

The culture evolution was followed by two methods, the dry biomass concentration and the concentration using the OD method, for a culture with a temperature between 34 and 37 ° C, and a light intensity of 40 W / m². The initial culture concentration, of this experience, was 0.15 g/l.

3.1.1. Determination of biomass concentration by the method of OD and of the dry biomass

Every day the concentration was measured through the OD and dry biomass method.

The OD is read by spectrophotometer with a wavelength of 750 nm. Thus, the equation obtained by the calibration curve gives the concentration in dry mass corresponding to the values of OD obtained (Appendix VI and VII). All points measured by this method and the corresponding concentration are represent in the Figure 13.

Using the dry biomass method was possible to verify the biomass evolution, from a concentration of 0.15 g / l, as can be seen in Figure 13.

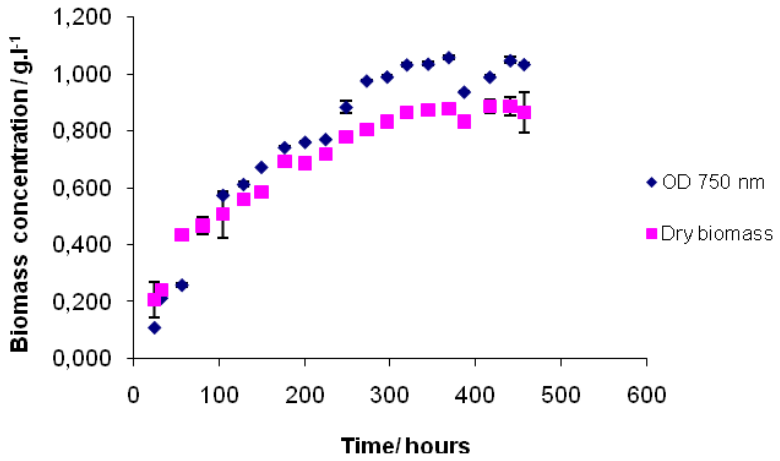


Figure 13- Evolution of biomass concentration of *Spirulina platensis* in time, using the methods of optical density and dry mass, at 40 W/m² , with a temperature between 34 and 37 °C.

Analyzing the curve that represents the dry biomass method, the *Spirulina platensis* concentration after 24 hours of starting the culture is 0.207 g/l. From these results, this

concentration changes over time in three phases. The first phase is presented by a rapid change in concentration until the 319th hour at 0.87 g / l. From that date on, the growth rate in dry biomass of *Spirulina platensis* decreases until it stabilizes. The last phase begins after stagnation of evolution, or when the concentration in *Spirulina platensis* is constant, because the light intensity is not sufficient to increase the concentration. So, light is absorbed at the surface and nothing goes in the center. Also, in this Figure it can be verified that three points have a larger standard deviation. This could be due to the fact that the culture is not homogeneous (1 start, 2 to change lamps, 3 at the end when the culture clusters on the reactor walls) and that has reflect in the different measures of dry weight.

By the OD method, it can be seen that this method reaches its maximum after 24 days, at 40 W/m², with a concentration of 1.049 g/ l, however, for the same interval of time, the dry biomass method reaches a concentration of 0.887 g/l.

The difference between the values obtained by both methods may be due to the size of the filaments. The more filaments are broken, the more they diffract the light, so their optical density is higher.

3.1.2. Determination of phycocyanin concentration

The follow-up of the concentration in phycocyanin in the photobioreactor has been carried out only on 15 days of culture, because at the beginning of culture, the concentration in biomass is very low. Therefore the measures have begun after the 3rd day up to the 23th day, when the biomass is more concentrated. The evolution of the concentration of phycocyanin during the growing period of the culture is shown in Figure 14 (Appendix VIII).

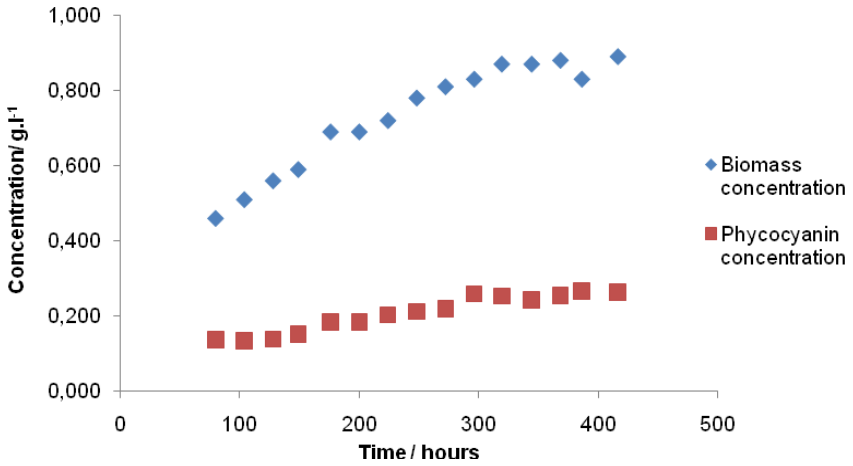


Figure 14 – Evolution of phycocyanin concentration in the bioreactor as a function of time, at 40W/m² and temperature between 34 and 37°C.

3.1.3. Produtivity and growth rate

The productivity of *Spirulina platensis* biomass is given by the formula *Danesi et al*. (2004), and the growth rate determination is given by de equation **(1.1)**, using an interval of time of [56, 368] hours.

Table 10 – Results of productivity and growth rate

$C_{initial}$ (g/l)	C_{final} (g/l)	Δt (days)	Average Productivity (g/l/day)	μ_{max} (h^{-1})
0.207	0.886	18	0.038	0.0024

3.2. Experiment 2

In this experience, it is intended to simulate a family production with a levy of 1/ 5 of the total reactor volume. So, in every day of dilution the amount withdrawn directly from the photobioreactor was 8 liters. Relatively to the quantity of sample used in the measurement for each method, it is the same quantity previously mentioned (200 ml).

It is also necessary to refer that this experience is a continuation of the previously conducted experiment. Thus, the initial concentration was 0.807 g/ l, at 40 W / m² and temperature between 34 and 37 ° C.

3.2.1. Determination of biomass concentration by the method of OD and of the dry biomass

For each day the reading of the OD at 750 nm was made. From the equation obtained by the calibration curve, it was obtained the relationship between the OD and the respective concentration of dry biomass (Appendix VI and VII).

Using the method of the dry biomass described previously, it was obtained the evolution of biomass concentration (Figure 15).

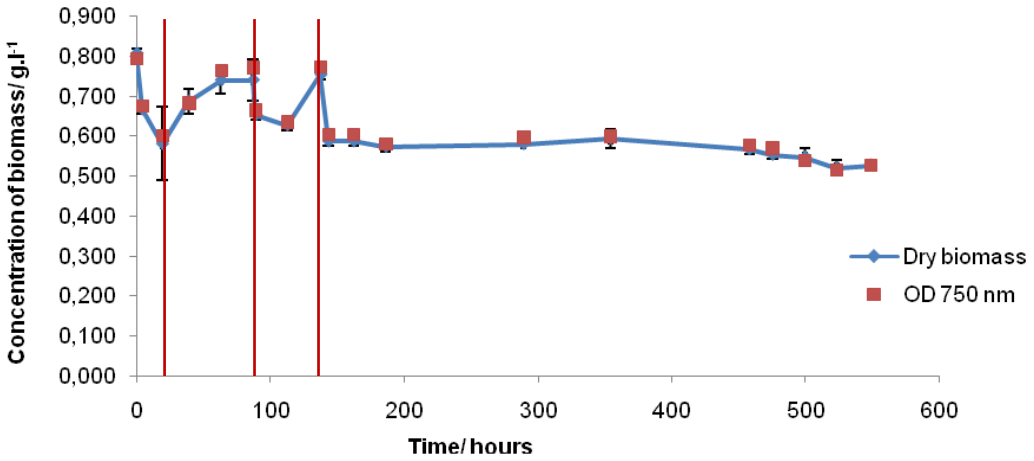


Figure 15 – Evolution of biomass concentration of *Spirulina platensis* in time, using the methods of optical density and dry mass in function of time, with 40 W/m² and temperature between 34 and 37°C (—: represents sample of 8 liter in 40 liter) .

Analyzing the illustration, it is verified that in the first two dilutions made in the culture, with initial concentrations of 0.807 g/l and 0.653 g/l, respectively, there is an accentuated decrease of the biomass concentration, due to the high disturbance made. After this, there is an increase in the concentration, as expected. The third dilution, with an initial concentration of 0.587 g / l, starts with an accentuated decrease of the concentration, however, in the following days this decrease continued. This event may have occurred due to the high amount of salt present in the culture medium, explained by the fact that the interval of time between the second and third dilution is reduced, leading to saturation of the culture and that is reflected by its inhibition. On the other hand, this phenomenon may have occurred due to the high pH, provoking the reduction of the culture growth as it can be seen from the Figure 4. This was provoked by the impediment of the measurement of the pH.

For the OD method, the same occurrence is viewed.

Among the results obtained for the concentration in both methods, it is verified that a divergence among these takes place.

3.2.2. Determination of phycocyanin concentration

During the following 19 days of culture, it was equally performed the measurement of the phycocyanin concentration, using the spectrophotometer at 620 nm and the equation of *Silveira et al.* (2007) (Appendix VIII). The evolution of phycocyanin concentration is demonstrated by the following Figure.

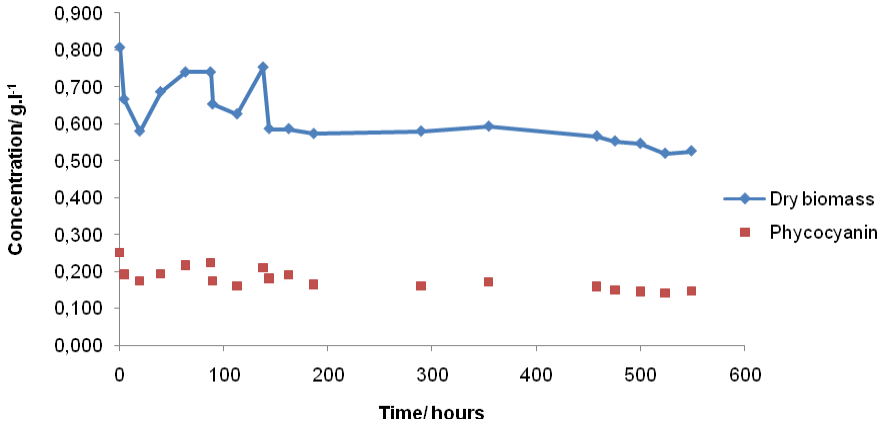


Figure 16 - Evolution of phycocyanin concentration and biomass concentration as a function of time, at 40 W/m² and temperature between 34 and 37°C.

3.2.3. Productivity and growth rate

Each dilution has a different growth of the biomass development. Thus, the growth rate was determined with an interval of time, for the first dilution, of [19, 87] hours, and for the second, of [112.5, 137.5] hours.

Table 11- Results of productivity and growth rate for the first two dilutions

Dilution	$C_{initial}$ (g/l)	C_{final} (g/l)	Δt (days)	Average productivity (g/l/day)	μ_{max} (h^{-1})
1	0.667	0.770	4	0.0258	0.0026
2	0.653	0.753	5	0.0200	0.0074

3.3. Experiment 3

For a better perception of light intensity influence in the development of the culture, it was necessary to appeal to the integration of a system of temperature regulation, in order to focus on light intensity of 100 W / m² (20 lamps) and maintaining the temperature at 35 ° C.

This experience is the continuation of the culture study began at the previous experience. Thus, the initial concentration is 0.527 g / l.

3.3.1. Determination of biomass concentration by the method of OD and of the dry biomass

To obtain the values for OD, it was used the spectrophotometer with a wavelength of 750 nm. From the calibration curve and the values obtained in the OD, it was possible to establish the relationship between the OD and the corresponding concentration of culture (Appendix VI and VII).

Using the dry biomass method, it was possible to obtain the culture evolution.

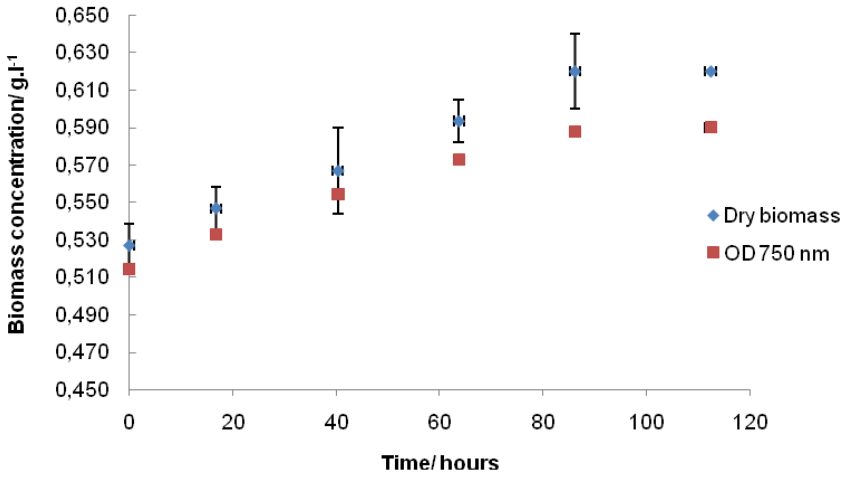


Figure 17 – Evolution of biomass concentration of *Spirulina platensis* in time, using the methods of optical density and dry mass in function of the time, at 100 W/m² and a temperature of 35°C.

This experience began with a concentration of *Spirulina platensis* to 0.527 g/ l. With the representation of the results, it was observed that the evolution of the concentration developed in two phases. The first phase corresponds to the accentuated evolution of the biomass growth after 86 hours the start of the experiment, reaching the concentration of 0.620 g/l. The second phase is the stabilization of the concentration evolution verified after further 26 h. This is due to the incapacity of the culture to absorb light.

For the OD method, the same was visualized. This method started with a concentration level of the 0.514 g/l and after 8 days a maximum concentration of 0.590 g/l was reached.

The difference in concentration obtained between the two methods is due to the "age" of culture. Since this culture was maintained for three experiences, the filaments of *Spirulina platensis* are more developed, consequently there is a greater absorption of light, decreasing its optical density.

3.3.2. Determination of phycocyanin concentration

During the experiences the follow up of phycocyanin concentration was conducted (Appendix VIII). Using the equation of *Silveira et al.* (2007), obtained the concentration of this pigment.

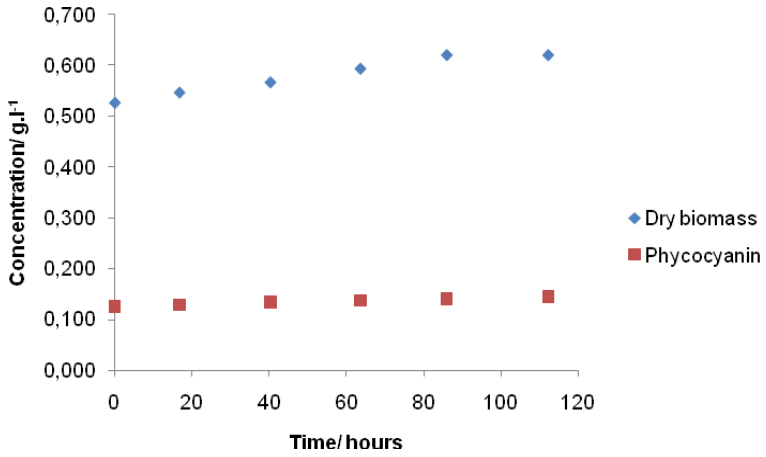


Figure 18 - Evolution of phycocyanin concentration and biomass concentration as a function of time, at 100 W/m² and with a temperature 35°C.

3.3.3. Productivity and Growth rate

The productivity of biomass is obtained through the equation of *Donesia et al. (2004).* For growth rate, this is obtained through the equation **(1.1)**, using an interval of time of [0, 86.1] hours.

Table 12 - Results for productivity and the growth rate

$C_{initial}$ (g/l)	C_{final} (g/l)	Δt (days)	Average Productivity (g/l/day)	μ_{max} (h^{-1})
0.527	0.620	8	0.0117	0.0019

3.4. Experiment 4

For this experience, it was necessary to reduce the capacity of the photobioreactor, 20 liters, due to a setback at the culture level. Thus, it was necessary to begin the experience from a lower concentration, 0.0525 g/l, consequentially it was necessary a low light intensity, 30 W / m², and a temperature of 33°C, during 48h, which is subsequently increased to 35 ° C.

3.4.1. Determination of biomass concentration by the method of OD and of the dry biomass

The values obtained by the OD method at 750 nm, using a spectrophotometer, were established by the concentration from the calibration curve (Appendix VI and VII) and by the monitoring of the culture growth.

To obtain the values for the different concentrations of the dry biomass, it was used the method previously described.

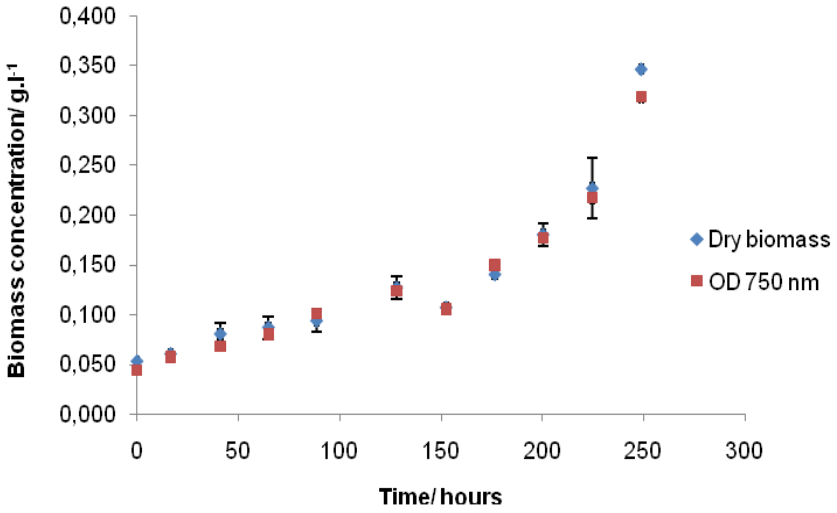


Figure 19 - Biomass concentration of *Spirulina platensis* versus time, using the methods of optical density and dry mass, in function of time, at 30 W/m² and with a temperature of 35°C.

The evolution of the biomass growth is in an exponential phase, started with a concentration of 0.0525 g/l, and not reaching the stage of stabilization. In the 128th hour of the experiment, it was necessary to control the pH by adding sulfuric acid (H_2SO_4). This procedure provoked a decrease in the biomass concentration. This is due to the violent contact between the culture and the acid, causing the inhibition of the culture during 24 h. After this event, culture incorporates its exponential growth.

For the OD method the same was visualized and it started with a concentration of 0.0445 g/l.

The differences between the two methods are smaller, because of the low concentration of the culture in this experience.

3.4.2. Determination of phycocyanin concentration

Due to the low concentrations, it was not possible to proceed of the phycocyanin concentration measurement as the values obtained for a wavelength of 620nm were negative.

3.4.3. Productivity and Growth rate

The productivity and growth rate are obtained by the equations by *Danesi et al.* (2004) and **(1.1)**, respectively. The interval of time used for the growth rate determination is [0, 248.8] hours.

Table 13 - Results for the productivity and grow rate

$C_{initial}$ (g/l)	C_{final} (g/l)	Δt (days)	Average Productivity (g/l/day)	μ_{max} (h^{-1})
0.053	0.330	14	0.0198	0.0063

4. Cultures with a variable light intensity

4.1. Experiment 5

This experience is the follow-up of the previous one, obtaining for that the total capacity of the photobioreactor with a dilution of the culture.

The aim of this experience is to verify the behavior of the culture growth, and the influence of the light intensity. So, this experience started with a concentration of 0.190 g/l, at 30W/m².

4.1.1. Determination of biomass concentration by the method of OD and of the dry biomass

By using the spectrophotometer, the measurement of OD was preceded at a wavelength of 750 nm (Appendix VII). Based on the calibration curve equation, it was possible to associate the values of the corresponding concentration (Appendix VI).

The dry biomass method was performed as previously described.

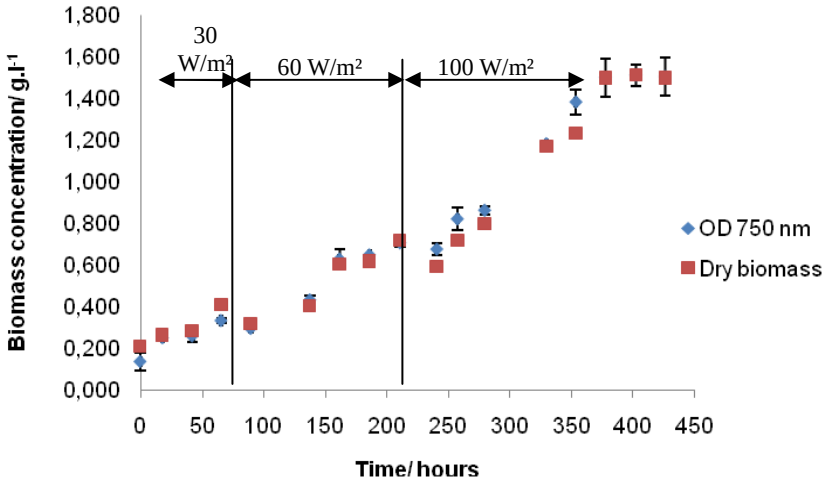


Figure 20 - Biomass Concentration of *Spirulina platensis* versus time, using the methods of optical density and dry mass, with a temperature of 35°C.

Along with this experience preceded the change of luminous intensity, having for that different exponential stages.

The difference between concentrations obtained by the OD and the dry biomass methods are smaller.

Throughout the study of the evolution of culture, it was also performed the salinity and total inorganic carbon measurement, using the methods mentioned previously.

In the following illustration, it is possible to verify that the salinity and total inorganic carbon decreases with the increase of biomass concentration. This was expected, since the salinity, which comes from the culture medium, was consumed during the growth of culture. For the total inorganic carbon, the increase of light intensity leads to an increase in the cells growth rate, leading to a

higher consumption of carbon, as it is shown in the initial and final concentrations, 0.300 g / l and 0.135 g / l, respectively.

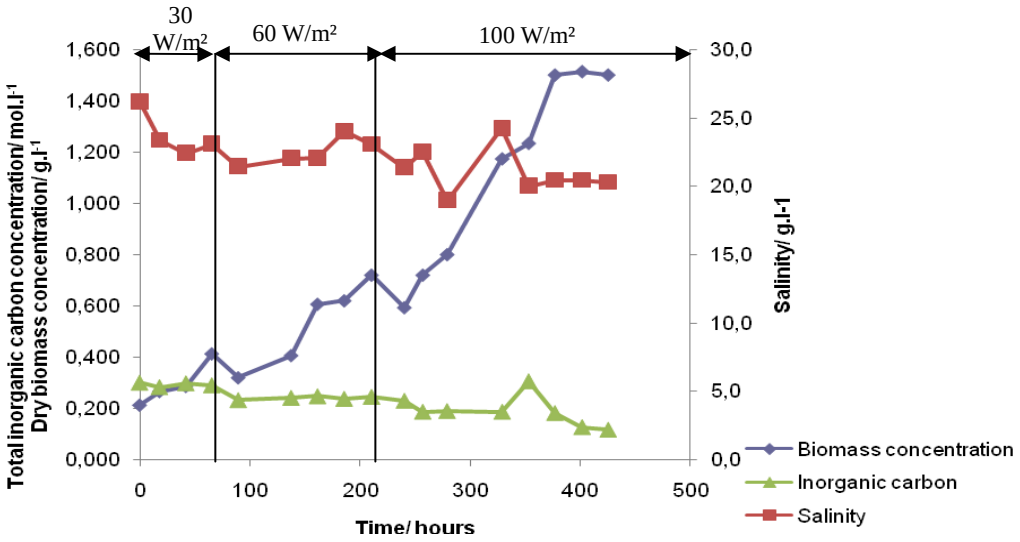


Figure 21 - *Spirulina platensis* biomass, the salinity and total inorganic carbon evolution in time, in each variation of light intensity, with a temperature of 35°C.

4.1.2. Determination of phycocyanin concentration

Using the spectrophotometer with a wavelength of 620 nm (Appendix VIII) and the equation of *Silveira et al. (2007)*, it was obtained the concentration of phycocyanin.

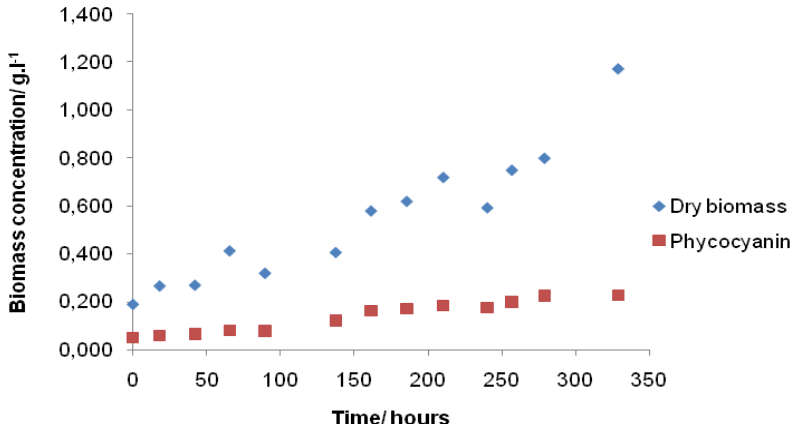


Figure 22 - Evolution of phycocyanin concentration and biomass concentration as a function of time, at different light intensity and with a temperature of 35°C.

The phycocyanin concentration has an exponential evolution, and it was not disturbed by the increase in light intensity as it was verified for the biomass concentration.

4.1.3. Productivity and Growth rate

The productivity and growth rate are obtained by the equations by *Danesi et al.* (2004) and **(1.1)**, respectively. For the growth rate determination it is used an interval of time for the light intensity of 30 W/m² of [0, 42.1] hours, for 60 W/m² of [137.6, 210.6] hours, and for 100 W/m² of [257.2, 425.5] hours.

Table 14 - Results of productivity and growth rate

$C_{initial}$ (g/l)	C_{final} (g/l)	Δt (days)	Average Productivity (g/l/day)	μ_{max} (h^{-1})
0,19	1,173	15	0,068	30 W/m²: 0,0079 60 W/m²: 0,0073 100 W/m²: 0,0060

5. Conclusion

The concentration and productivity of *Spirulina platensis* depends on culture conditions, such as: the state of the filaments, light intensity, agitation speed and the pH of the medium. The attachment of filaments on the inner bioreactor walls obstructs the propagation of light, which provokes a decrease of luminous flux at the bioreactor center.

The use of a constant light intensity throughout the growing period may be sufficient to start with a low concentration of biomass, but increasing the concentration of biomass during cultivation may reduce its intensity, which will consequently reduce the *Spirulina platensis* productivity in the bioreactor.

For different light intensities, the statement is highlighted, as can be verified from experience 5 (Table 14). In this experience it can be concluded that with the increasing of light intensity, the productivity decreases, because the growth rate decreases with the time of culture.

Relatively to the results obtained in all experiences carried out, it was verified that the productivity obtained in the experiences is lower when compared to the literature (Table 4), in the same operating conditions.

The results obtained from phycocyanin concentration, in all the experiments realized, have higher values than the ones presented in literature. This is because of the lamp type used in the photobioreactor.

General conclusion and future perspectives

Studies in this work have been divided into two parts. The first one focuses on the design of a photobioreactor for the *Spirulina platensis* production for family consumption. The second part is for the study of culture aspects and biochemical dosage of *Spirulina platensis*.

The design of the photobioreactor allowed responding to a need, allowing the user to produce its own culture of *Spirulina spp.*.

Using the design methods, there are actually four solutions that could correspond to the user need. These solutions should be, in the future, analyzed with greater accuracy, at the level of the reactor dimensions, the geometry adopted, the lamp type, the agitation system, and the material to be used.

Two technological aspects were analyzed in order to guide the future photobioreactor conception, the type of agitation and the study of the lighting system. From this analysis it was possible to verify that the agitation system, which is part of a reactor of 10 liters, is a bubbling system. For the lighting system study, it was possible to perceive the importance of the position of the lighting system in a reactor.

The culture study of the *Spirulina platensis* was accomplished through the execution of five experiences for verify the biomass concentration in conditions of different light intensity. For that, two methods were used: the indirect method by measuring the optical density, and a direct method by the measurement of dry biomass concentration. The first method is a little delicate, since the filaments of *Spirulina platensis* are not stable, so there must be a quick reading of the absorbance of the curve.

Under conditions of constant pH, temperature and agitation speed, the reproducibility in *Spirulina platensis* depends on the quantity of light received by the filaments in the photobioreactor. Indeed, the attachment of filaments in the internal photobioreactor walls decreases the light intensity inside propagation. In addition, the opacity of the cylindrical tube used for agitation presents an obstacle to the arrival of light to the *Spirulina platensis* mass. One of the improvements that have been envisaged is the use of an agitation system around the internal walls to prevent the attachment of filaments and also the use of a transparent cylindrical tube to allow the homogenization of the light along the photobioreactor.

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Appendix I – Description of the Photosynthesis: "Z-scheme"

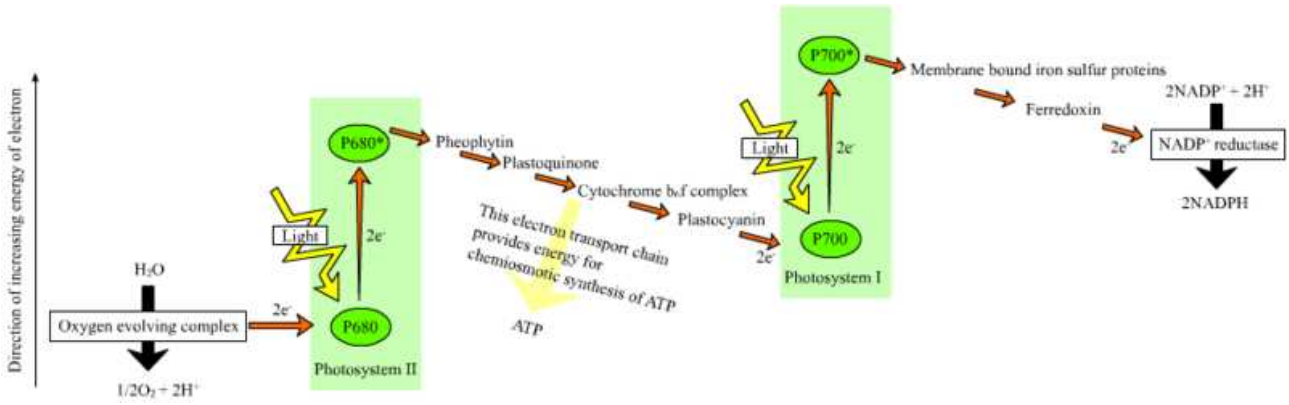


Figure 23 - Photosynthesis process: "Z-Scheme"

The photosynthesis start with the simultaneous excitation of pairs of special reaction center chlorophyll a, molecules labeled as P700 (PSI) and in P680 (PSII) with phycocyanins molecules. The phycocyanins molecules are aggregated in phycobilisomes.

The excitation energy comes from directly absorption of light or by energy transfer from adjacent pigment molecules into protein complexes called "antennas". These "antenna" pigment molecules (chlorophylls, carotenoids and phycocyanin) absorb light energy and transmit by inductive resonance from one molecule to the next, finally to the reaction center.

When a chlorophyll molecule in the core of PSII obtains sufficient excitation energy, an electron is transferred to the primary electron-accepter molecule, pheophytin, throught a process called photoinduced charge separation. These electrons pass through an electron transport chain, which leads to a chemiosmotic potential across the membrane.

During the electron transport chain, an ATP syntheses enzyme uses the chemiosmotic potential to make ATP during photophosphorylation, where NADPH is the final product of the redox reaction in the electron transport chain.

When the electron reach the photosystem I (PSI), it is again excited by light energy absorbed. A second electron carrier accepts the electron, which again is passed down lowering energies of electron acceptors.

The excited electrons lost from PSI are replaced from electron transport chain by plastocyanin.

The source of electrons in green-plant and cyanobacterial photosynthesis is water. Two water molecules are oxidized by four successive charge-separation reactions by PSII to yield a molecule of O_2 and four H^+ *(Govindjee, 2000).*

Appendix II – Photobioreactores technology

Based on the literature was possible to elaborate the following table in way to verify the different geometry types, the position of the lighting system and the type of agitation system, for cultures of *Spirulina platensis*, and others algae's.

Table 15 - Diagrams of different photobioreactors of literature.

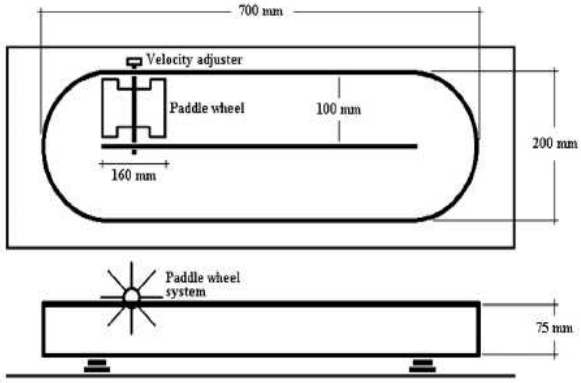
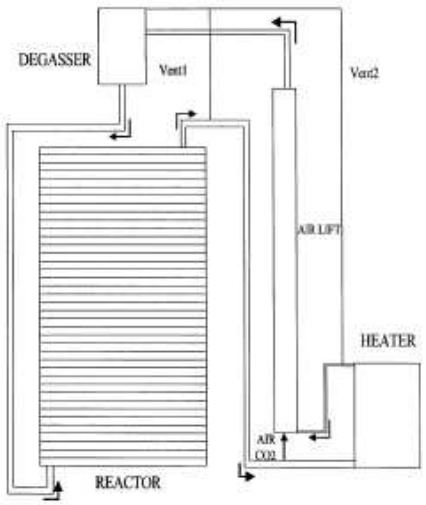
Photobioreactor	Characteristics
 **Figure 24 -** Diagram of the open raceway pond (Radmann *et al.*, 2007).	Algae: *Spirulina platensis* Effective volume: 6 liter Photobioreactor material: Acrylic Surface of light exposed: ~0.14 m² Type of lamps: fluorescent lamps (Osram, daylight-type) Light intensity of each lamp: 3000 lux Temperature: 30 °C Type of stirring: paddle-whell Rotation: 18 revs/ min Productivity: 0.028 to 0.046 g/ l /day
 **Figure 25 –** Diagram of helical photobioreactor (Travieso *et al.*, 2001)	Algae: *Spirulina platensis* Total volume: 21 liter Photobioreactor material: PVC Surface of light exposed: 1.32 m² Type of lamps: fluorescent lamps Total light intensity: 70.15 µmol.m^{-2}.s^{-1} (in first 250 h); 156.64 µmol.m^{-2}.s^{-1} (after) Temperature: 28 to 30°C Type of stirring: airlift Flow rate: 19.28 cm^{3}.s^{-1} Productivity: 0.40 g/ l /day

Table 16 - Diagrams of different photobioreactors of literature (continuation).

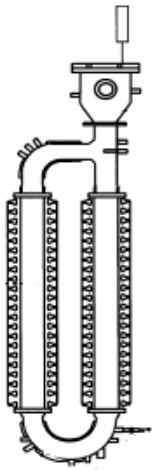
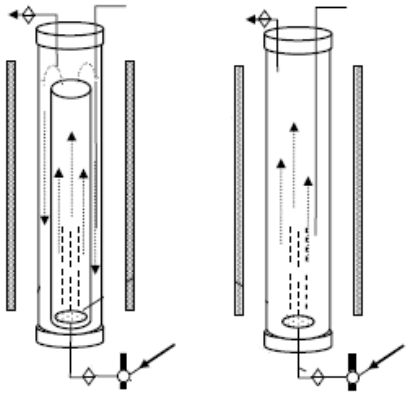
Photobioreactor	Characteristics
 **Figure 26-** Airlift photobioreactor (Morist *et al.*, 2001).	Algae: *Spirulina platensis* Total volume: 77 liter Type of lamps: halogen lamps (Osram Sylvania, 12 V, 20 W) Total light intensity: 133 $W.m^{-2}$ Temperature: 36 ± 1°C Type of stirring: airlift Flow rate: 1.5 $l.min^{-1}$ Productivity: 0.13 g/ l /day
 **Figure 27 –** Diagram of airlift(a) and bubble (b) photobioreactor (Once *et al.*, 2008)	Algae: *Spirulina platensis* Volume effective: 1.5 liter Photobioreactor material: Glass Type of lamps: fluorescent tubes (Philips TLD/54, 18 W) Number of lamps: 2 Total light intensity: 5200 lux Temperature: 25 ± 1°C Type of stirring: airlift (a) and bubble (b) Flow rate: 1.5 $l.min^{-1}$

Table 17 - Diagrams of different photobioreactors of literature (continuation).

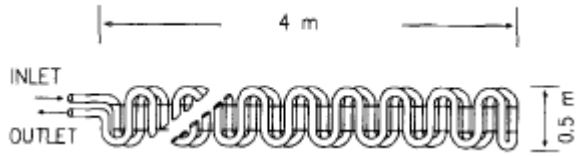
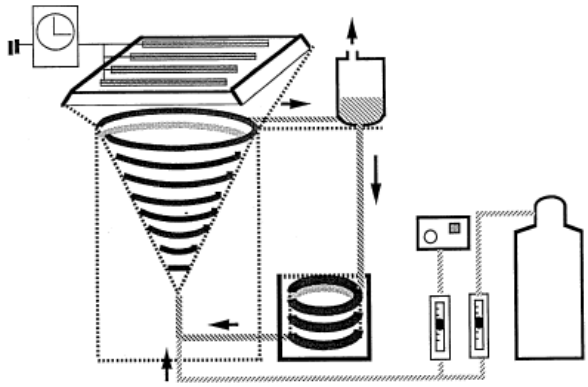
Photobioreactor	Characteristics
 **Figure 28 –** Diagram Strengly airved reactor (Carlozzi and Torzillo, 1996)	Algae: *Spirulina platensis* Total volume: 38 liter Photobioreactor material: Plexiglass Light: Solar Total light intensity: 1735 ± 30 µmol.m^{-2}.s^{-1}; 1300 ± 100 µmol.m^{-2}.s^{-1} Temperature: 35 °C Type of stirring: Pump
 **Figure 29 –** Diagram of Cone-Shaped helical tubular photobioreactor system (Watanabe and Hall, 1996).	Algae: *Spirulina platensis* Total volume: 6.23 liter Photobioreactor material: PVC Surface of light exposed: 0.651 m² Type of lamps: compact fluorescent cool white lamps (Osram ,colour appearance21, 55W) Number of lamps: 4 Total light intensity: 546.8 µmol.m^{-2}.s^{-1} Temperature: 36 ± 1 °C Type of stirring: airlift Flow rate: 0.3 l.min^{-1} Productivity: 0.51 g/ l /day

Table 18 - Diagrams of different photobioreactors of literature (continuation).

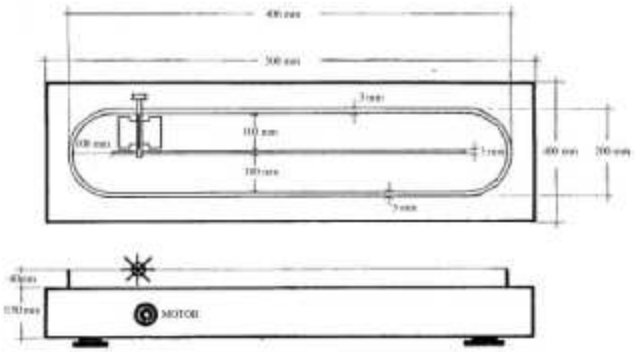
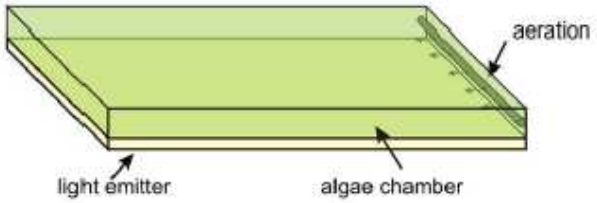
Photobioreactor	Characteristics
 **Figure 30 –** Schematic representation of mini-tanks (Pelizer *et al.*, 2003)	Algae: *Spirulina platensis* Total volume: 5 liter Photobioreactor material: Acrylic Surface of light exposed: ~0.081 m² Type of lamps: fluorescent lamps Total light intensity: 3500 lux Temperature: 30 °C Type of stirring: rotary agitator Rotation: 180 rpm Productivity: 0.67 to 0.76 g/ l /day
 **Figure 31 –** Schematic diagram of a photobioreactor tank (Ganzer and Messerschumid, 2009)	Algae: *Spirulina platensis* Effective volume: 2.4 liter Surface of light exposed: 0.1 m² Type of lamps: LED Light intensity: 1800 µmol.s^{-1}.m^{-2} Type of stirring: air injection

Table 19 - Diagrams of different photobioreactors of literature (continuation).

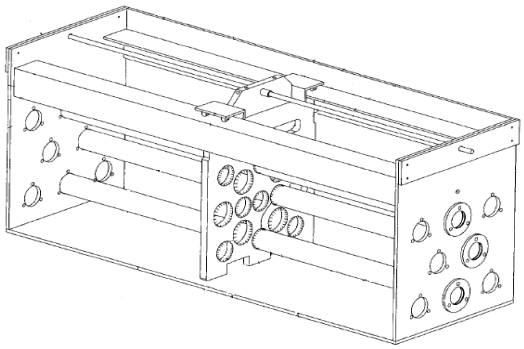
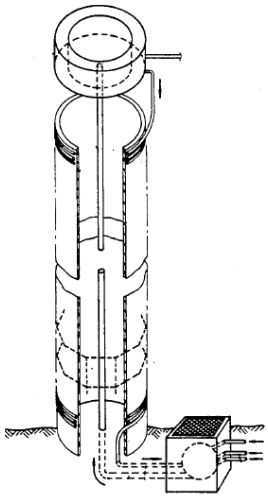
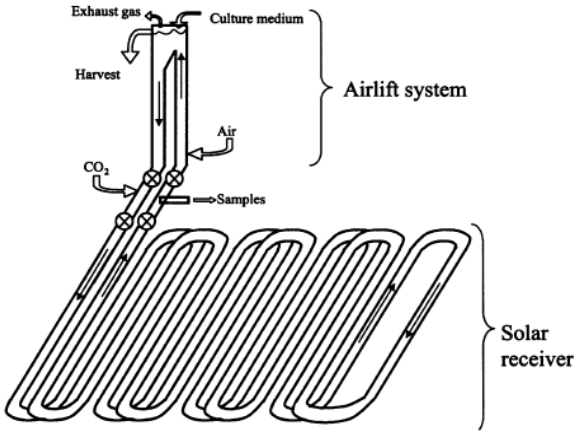
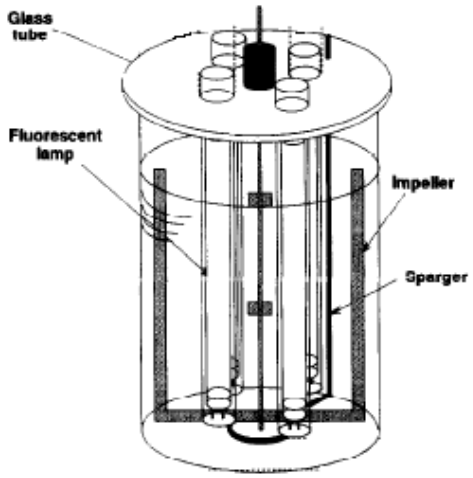
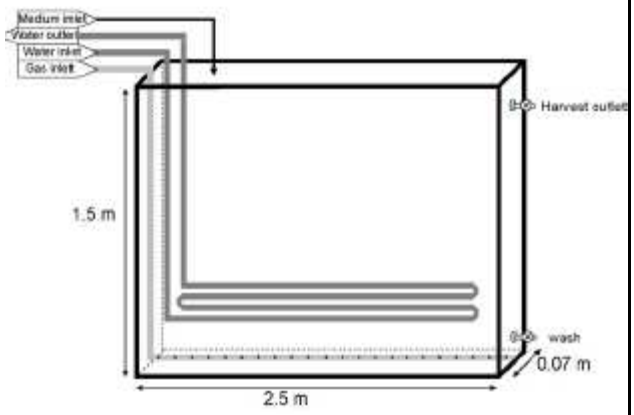
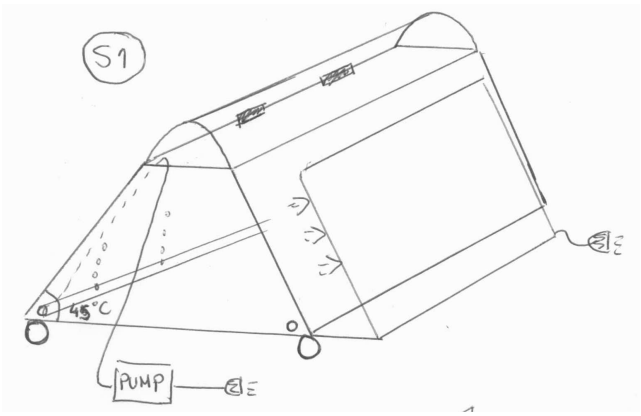
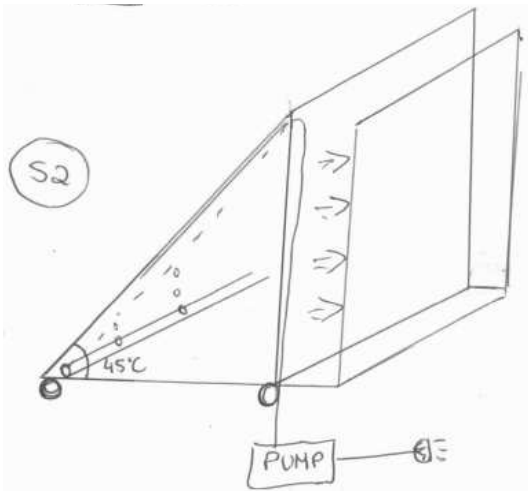
Photobioreactor	Characteristics
 **Figure 32 –** Photobioreactor (Dutil, 2003)	Algae: *Spirulina platensis* Effective volume: 3.1 m^3 Total volume: 7m^3 Photobioreactor material: Acrylic Surface of light exposed: 2.88 m^2 Type of lamps: Cool white fluorescent lamps (80W, each) Number of lamps: 8
 **Figure 33** -Core Photobioreactor (Robinson *et al.*, 1992)	Algae: *Spirulina platensis* Total volume: 1.3 liter Photobioreactor material: Polyethylene Surface of light exposed: 100 m^2 Type of lamps: fluorescent lamps Temperature: 35 °C Type of stirring: air injection
 **Figure 34 -** Tubular photobioreactor (Molina *et al.*, 2000)	Algae: *Phaeodactylum tricornutum* Total volume: 0.2 m^3 Photobioreactor material: Plexiglass Surface of light exposed: 12 m^2 Light: Solar Temperature: 20 °C Type of stirring: airlift Productivity: 1.90 g/ l/ day

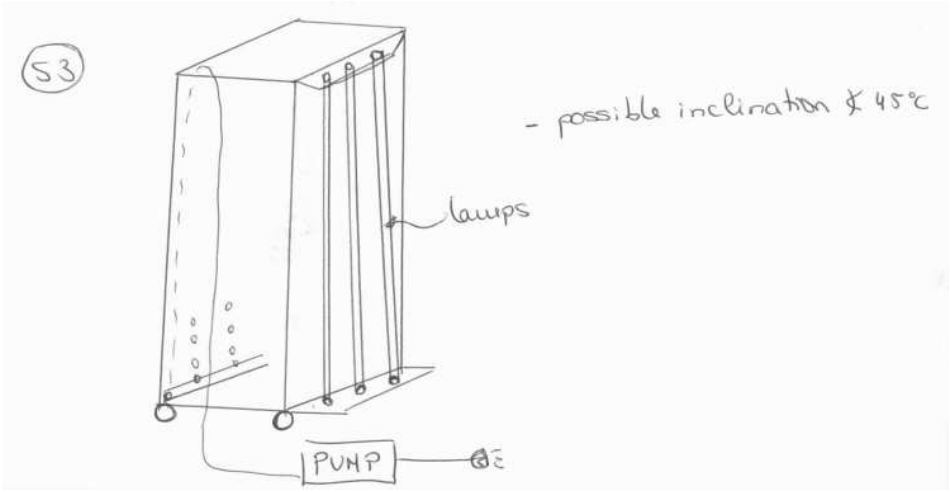
Table 20 - Diagrams of different photobioreactors of literature (continuation).

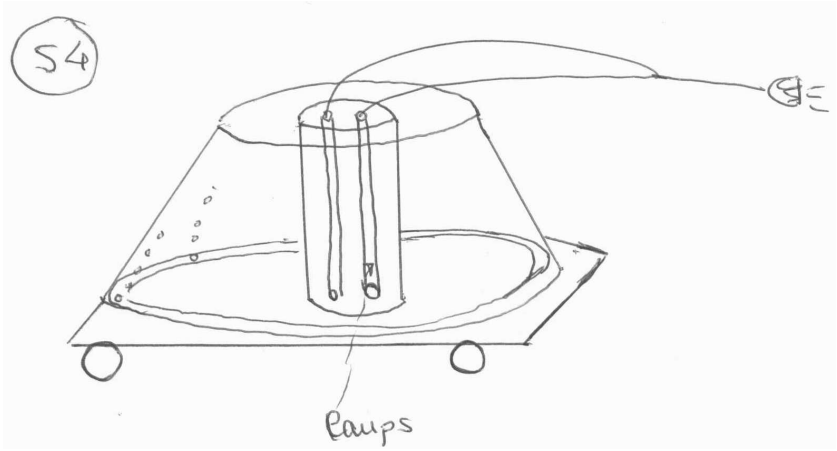
Photobioreactor	Characteristics
 **Figure 35 –** Stirred tank photobioreactor (Ogbonna *et al.*,1996)	Algae: *Chlorella pyrenoidosa* Total volume: 2.6 liter Photobioreactor material: Plexiglass Type of lamp: fluorescent (4W) Light intensity: 163 µmol.m^{-2}.s^{-1} Type of stirring: Stirred system Rotation: 250 rpm
 **Figure 36 -** Flat panel photobireactor (Sierra *et al.*,2007)	Algae: All species of microalgae Total volume: 250 liter Photobioreactor material: Polyethylene Light: Solar Type of stirring: Bubbles

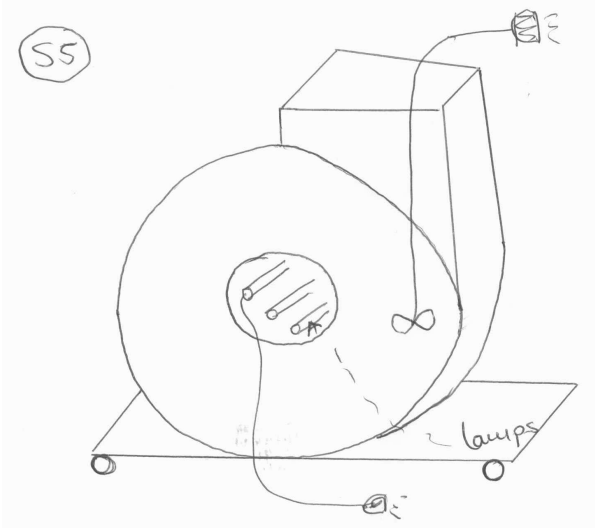
Appendix III - Solutions

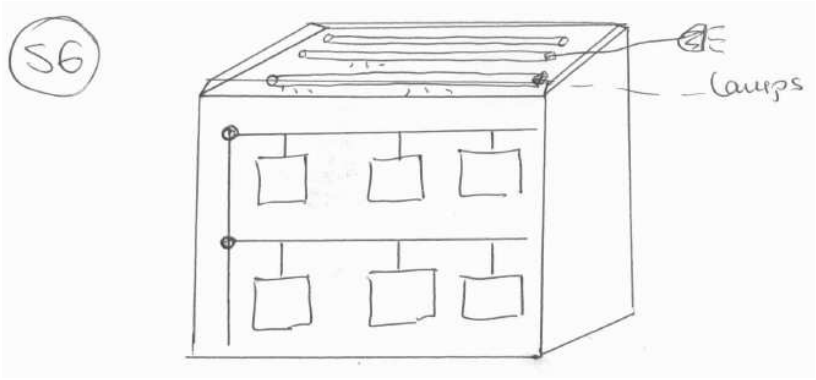


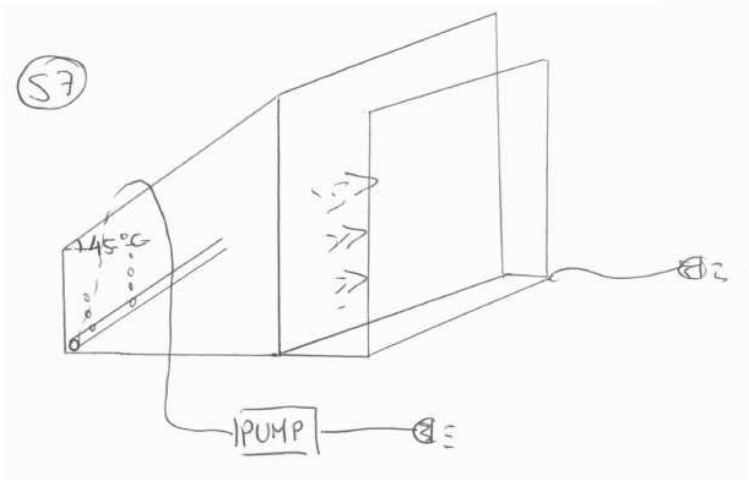


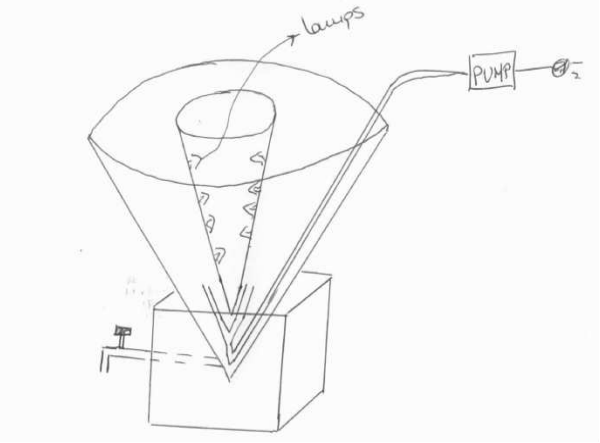





S5
lamps


S6
lamps


S7
45°C
PUMP


lamps
PUMP

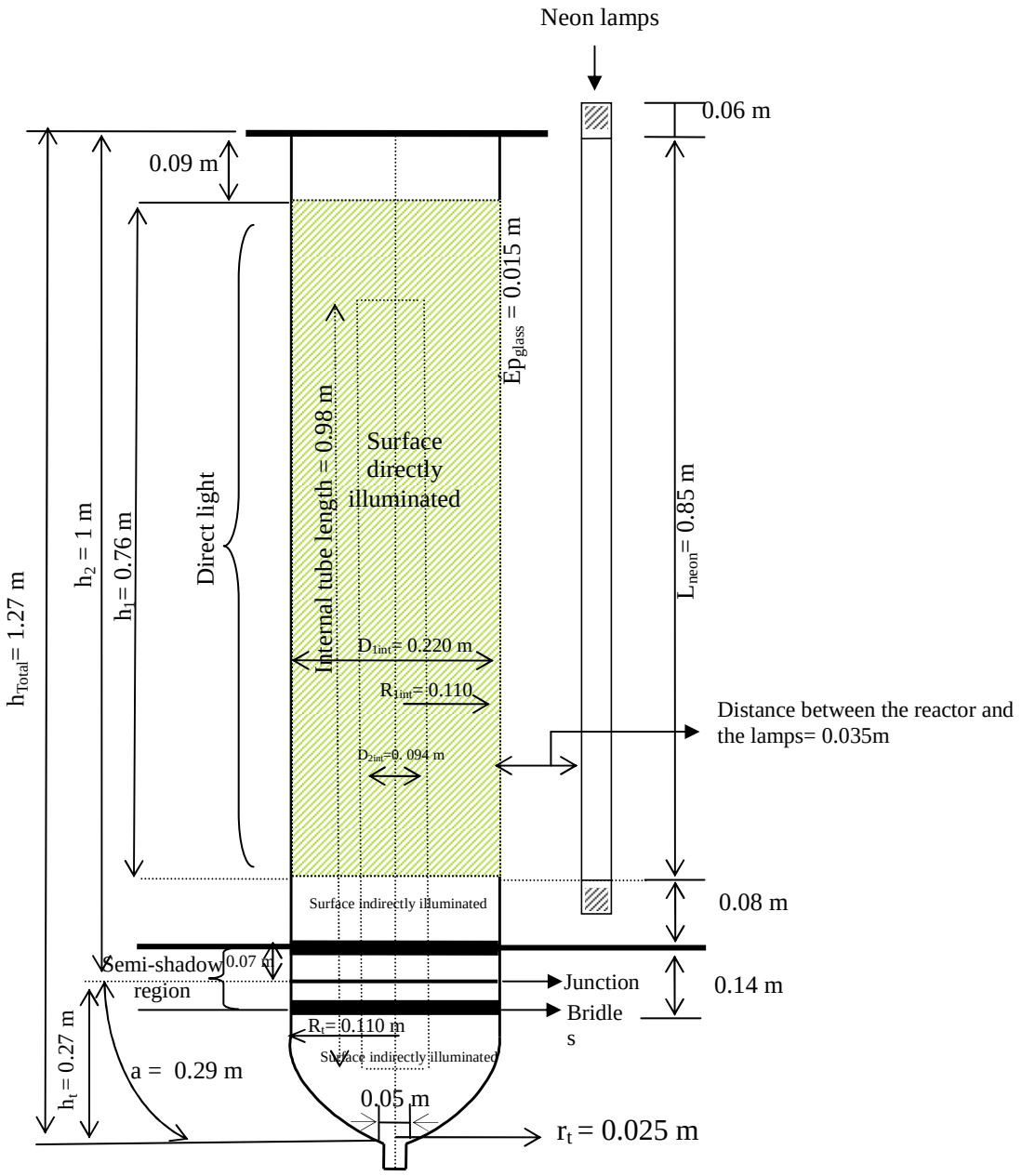
Appendix IV – Selection of Solutions

Using the results from de functional hierarchy, it was accomplished the elimination of the proposed solutions.

Table 21 - Comparation between the several solutions and the functions.

Functions / Solution	F_1 (6.04 %)	F_2 (5.49 %)	F_3 (8.24 %)	F_4 (18.1 %)	F_5 (5.49 %)	F_6 (2.75 %)	F_7 (3.30 %)	F_8 (13.2 %)	F_9 (9.89 %)	F_{10} (13.2 %)	F_{11} (13.7 %)	F_{12} (0.549 %)	**Total**	**%**
S_1	2	3	3	2	4	3	3	3	3	3	3	2	2.81	11,4
S_2	4	3	3	4	4	4	4	4	4	4	3	4	3.72	15,2
S_3	3	3	3	4	3	4	3	4	3	4	4	3	3.61	14,7
S_4	3	4	3	3	4	3	4	3	4	3	4	4	3.38	13,8
S_5	3	3	3	3	3	3	3	3	3	4	4	3	3.27	13,3
S_6	3	4	4	3	2	2	2	2	3	3	2	2	2.75	11,2
S_7	3	2	3	3	4	3	3	3	3	3	2	3	2.86	11,6
S_8	2	2	2	3	2	2	2	2	2	2	2	2	2.18	8,9
												Total	24.58	100

Appendix V – Dimensions of the LAGEP photobioreactor



1. Dimensions :

1.1 Cylinder

D_{1int} = Interne Diameter of the cylinder = 0.22 m

R_{1int} = Internal radius of the cylinder = 0.11 m

h_2 = Height of cylinder = 1 m

D_{2int}= Inside diameter of the inner tube PVC = 0.094 m

Length of PVC inner tube = 0.98 m

1.2 Trunk cone

R_t = Radius upper truncated cone = 0.11 m

r_t = Radius lower truncated cone = 0.025 m

a = Apothem of truncated cone = 0.29 m

h_t = Height of truncated cone = 0.27 m

2. Volume :

Spirulina volume in the reactor = $V_{Spirulina}$ into cylinder + cone V_{tronc}

$V_{Spirulina}$ into cylinder = $\pi R_{1int}^2 (h_2 - 0.09) = 34.59$ L

cone $V_{tronc} = \pi h_t /3 (R_t^2 + r_t^2 + R_t r_t) = 4.37$ L

Spirulina volume in the reactor = 34.59 L + 4.37 L = 38.96 L

3. Surface illuminated:

3.1 Total external surface of the reactor

S total reactor ext = S lateral vertical cylinder + Supper disk + Struncated cone

$= 2 \pi R_{1int} (h_1 + 0.08m) + \pi R_{1int}^2 + \pi a (R_t + r_t) = 0.5805m^2 + 0.038m^2 + 0.1229m^2$

$= 0.7414\ m^2$

3.2 Surface directly radiate

S direct radiation

= S cylinder side face directly exposed neon + S higher air disk exp

$= 2 \pi R_{1int} h_1 + \pi R_{1int}^2$

$= 0.5632\ m^2$

3.3 Surface indirectly radiate

S indirect radiaction = Sexternal truncated cone + Scyl indirect illuminated neon in below (h=0,08m)

$= \pi a (R_t + r_t) + 2\pi R_{1int} (0.08)$

$= 0.1229\ m^2 + 0.0552\ m^2$

$= 0.178\ m^2$

3.4 Surface illuminated directly and indirectly

= 0.5632 m² + 0.178m² = 0.7412 m²

3.5 Fraction illuminated surface

Fraction illuminated surface directly over $S_{externe}$ total culture medium = 0.5632 m²/ 0.7414 m² x 100 = 75.96 %

4. Airflow used:

Q_{min} = 10.6 l/h = 1.766 x 10^{-4} m^3/min = 2.9 x 10^{-6} m^3/s
Q_{max}= 26.5 l/h = 4.416 x 10^{-4} m^3/min= 7.36 x 10^{-6} m^3/s

4.1 Speed up = $\dfrac{Q \min and \max}{\dfrac{\pi D_{2\,int}^2}{4}}$

for Q_{min}, $V_{ascendante\ min}$ = 4.18 x10^{-4} m/s
for Q_{max}, $V_{Ascendante\ max}$ = 1.06 x 10^{-3} m/s

4.2 Speed downlink = $\dfrac{Q \min and \max}{\dfrac{\pi}{4}(D_{1int}^2 - D_{2\,int}^2)}$

for Q_{min}, $V_{descendante\ min}$ = 9.3x10^{-5}m/s
for Q_{max}, $V_{descendante\ max}$= 2.3 x10^{-4} m/s

5. Lamps :

20 neons :

- 15 neon white light spectrum absorption maximum 610 nm, 30W
- 5 solar-type neon Biolux, 30W

Appendix VI – Calibrations Curves

The calibration curves are obtained by the dilution results from each culture.

The following Table and illustration, represent de measurement of biomass concentration with the respectively OD at 750 nm.

Table 22 – Results for the calibration curve, for each experience.

Experiment 1,2 and 3		**Experiment 4**		**Experiment 5**	
Concentration/ g.l^{-1}	**$OD_{750\ nm}$**	**Concentration/ g.l^{-1}**	**$OD_{750\ nm}$**	**Concentration/ g.l^{-1}**	**$OD_{750\ nm}$**
0.069	0.1183	0.330	0.8105	0.300	0.5463
0.135	0.2207	0.264	0.6869	0.225	0.4312
0.207	0.3412	0.198	0.5155	0.150	0.2768
0.276	0.4881	0.165	0.4350	0.075	0.1473
0.345	0.5706	0.132	0.3531	0.038	0.0756

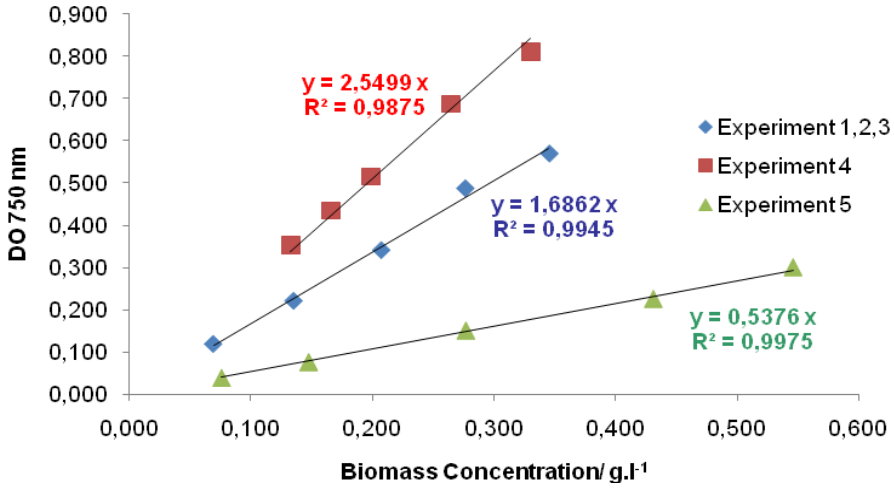


Figure 37- Calibration curve for each experience

Appendix VII - Measuring the biomass concentration of *Spirulina platensis* by optical density at 750nm

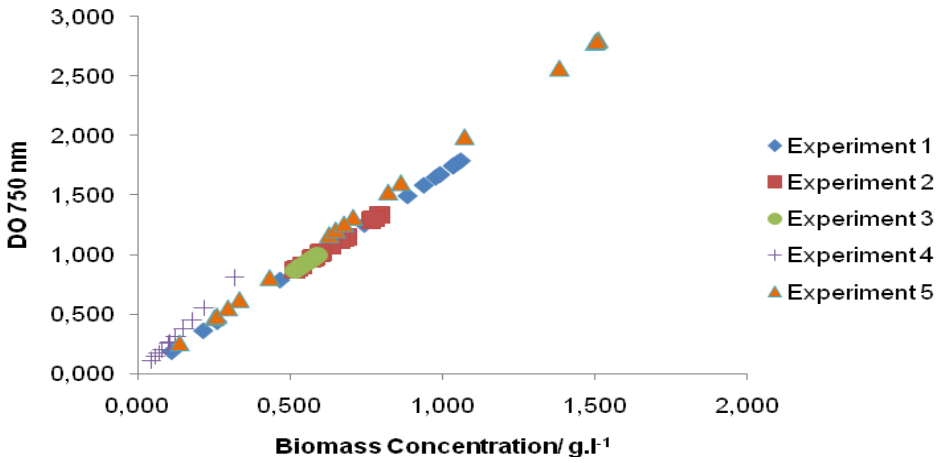


Figure 38- Representation of the OD and the corresponding concentration of *Spirulina platensis* from the calibration curve.

Appendix VIII – Measuring of the OD in *Spirulina platensis* at 620 nm

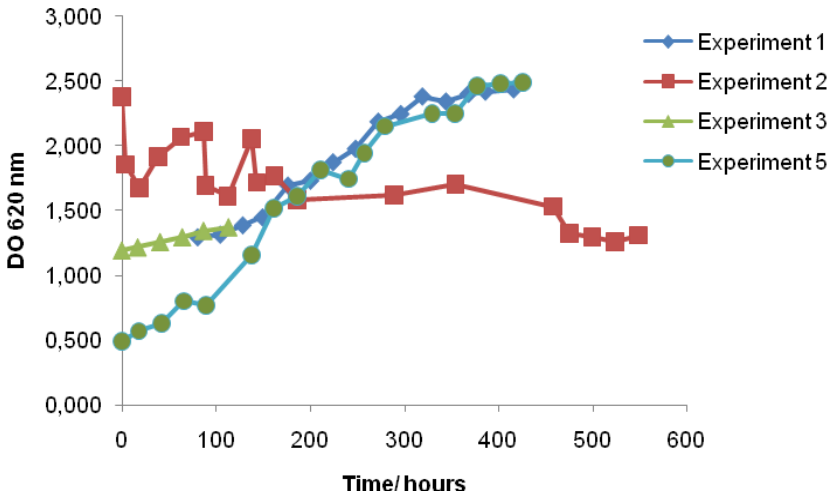


Figure 39 - The optical density of *Spirulina platensis* at 620 nm in function of time, for each experience.