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*Use of variable intensity LED illuminator to
optimize microalgal growth in a planar
photobioreactor*

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Climate change is real.

The science is compelling.

And the longer we wait,

the harder the problem will be to solve.

(John Forbes Kerry)

ABSTRACT

The possible use of microalgae as a new alternative for biogas upgrading has already been discussed in various precedent studies. Apart from this, microalgae have numerous applications, that make their cultivation economically advantageous. So, the purpose of this work is to identify the best microalgal accretion conditions, using an experimental planar photobioreactor and LEDs with variable intensities as light source. Two different illumination intensities were used during the experiment: 33 and 57 PAR. Observing trends of pH, CO₂, oxygen, nitrates and temperature, it is possible to monitor microalgal production and respiration. Furthermore, a correlation between optical density (OD) and light intensity was found, in order to evaluate microalgae growth under specific light conditions. So, given the biomass growth curve as a function of time, it is possible to perform a proportion to identify the value of the optical density resulting from the application of a luminous intensity of 1 PAR. This could be used in the future to proportionally calibrate the light intensity related with biomass density of PBR.

According to what has been identified in the literature, this type of research is absolutely innovative: in fact, it is difficult to find other experiments in which LEDs have been used, achieving the results obtained in this research. Moreover, in the consulted articles, no feedback was found regarding the correlation between light intensity and optical density: this is therefore the most important result of the whole work; it constitutes a fundamental starting point for future researches.

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1. INTRODUCTION

During the second half of the 20th century, a rapid growth caused some changes about the utilization of raw materials. In particular, a new importance was given to petroleum and natural gases, to the detriment of hard coal. However, it is clear that all these materials are limited and not renewable resources. Non-uniform distribution of these sources caused geopolitical problems too. The exhaustion of raw materials provoked several crises in the past, because of the dependence of economical world on oil. Moreover, the use of petroleum and natural gases provokes unwanted effects, such as acid rains and global warming, with consequences like climate change. At last, transport and energy producing industry are the main responsible of greenhouse gas emissions in the European Union: they produce, respectively, more than 20% and 60% on the total of these emissions. One of the most important greenhouse gasses is *carbon dioxide*, CO₂.

In the last years, global attention focused exactly on the problem linked to CO₂ emissions. In order to reduce this gas emission, and to limit its consequences, several technologies have been developed. Although these technologies may be different, they are based mostly on carbon capture and sequestration.

1.1 Microalgae CO₂ bio-fixation

There are many techniques for CO₂ sequestration, that can be classified in three main categories: physical, chemical, and biological. The first one refers to the processes that inject highly concentrated CO₂ into deep ocean, aquifers or depleted oil/gas wells. On the other hand, chemical fixation involves CO₂ immobilization using adsorption material, followed by alkaline-mediated neutralization leading to the formation of carbonates or bicarbonates (Zhou *et al*, “Bio-mitigation of carbon dioxide using microalgal systems: Advances and perspectives”). Among biological techniques, microalgae-based sequestration system has an increasing role: it is estimated that producing 100 tons of microalgal biomass fixes roughly 183 tons of carbon dioxide. (Razzak *et al*, “Integrated CO₂ capture, waste water treatment and biofuel production by microalgae culturing - A review”). In fact, microalgae can sequester low concentration CO₂ from air, or high concentration from CO₂ from point and stationary sources, such as coal burning power plants. Moreover, microalgae are able to fix CO₂, simply using sunlight, with a photosynthetic efficiency that is 10 – 50 times higher than terrestrial plants (Zhou *et al*. “Bio-

mitigation of carbon dioxide using microalgal systems: Advances and perspectives”). All the main advantages and disadvantages of this system are reported below:

Category	Method	Mechanism	Prospects	Disadvantages
Biological	Microalgae-based sequestration	Utilization of CO ₂ via microalgal photosynthesis	1) High photosynthetic efficiency	1) Sensitive to toxic substances in exhaust gases
			2) Efficient in low-concentration CO ₂ sequestration	2) Not very cost-effective for photobioreactors construction and algal biomass harvesting
			3) Faster sequestration rate than higher plants	
			4) Do not compete with crops for arable land	
			5) Co-production of food, feed, fuel, fine chemicals, etc.	

Table 1: Advantages and disadvantages of microalgae for CO₂ sequestration

The main sources of carbon dioxide emission can be classified as stationary, mobile or natural. Among the stationary ones, industrial processes play a central role. The ones that most contribute to increasing atmospheric CO₂ concentrations are the electrical energy generating plants (such as carbon and fossil fuel power plants, whose flue gases can be treated by microalgae) (Yun *et al.*, 1997), hydrogen and ammonia production plants, cement factories and kilns, and fermentative and chemical oxidation processes. The gas extracted from wells can be treated too. CO₂ emissions are also generated in residences, buildings and commercial complexes, but also by passenger and cargo transport, including cars, trucks, buses, planes, trains and ships. Moreover, human and animal metabolism, plant and animal degradation and

volcanic and oceanic activities too, contribute in CO₂ increasing. However, the use of microalgae-based sequestration system is restricted just to the industrial stationary emissions.

Another way for decrease the use of petroleum is its substitution. In many applications it is possible the use of biofuels: biogas is one of the most interesting one. Biogas is one of the main products of anaerobic digestion, made by methanogen or anaerobic organisms. With the term biogas we refer to a mixture of gas, produced from organic matter (like agricultural waste, plant material, green or food waste) in the absence of oxygen. Biogas can be used like biofuel, in many applications, like electricity production on sewage works, gas-grid injection or in transport. Biogas is roughly 55% methane, 45% carbon dioxide with trace elements of H₂S: so, it is inadequate for use in machinery. For this reason, it is necessary a process of purification, composed by subsequent steps, to obtain a final, clean biomethane. This process is called biogas upgrading. Its purpose is the elimination of every trace of hydrogen sulphide (because it can destroy mechanisms), water and particulate (if present), and carbon dioxide. The biogas CH₄ concentration should be upgraded to at least 90% (vol.%) to meet the standard for vehicles engine and natural gas pipe line systems. Many biogas upgrading techniques have been used in the past, such as absorption of liquids with physics/chemical adsorbent, membranes separation, pressure swing adsorption, and cryogenic separation. Although these methods are acceptable at biogas upgrading process, they usually need high costs and also consume a relative high quantity of energy during treating process. Most of these techniques also need complex operating systems, and produce undesirable end products, that require other processes or give secondary pollution. Furthermore, using these techniques, the CO₂ removed from the raw biogas, is normally discharged into the atmosphere, as greenhouse gas. In addition, most of these methods need a preventive H₂S removal.

In order to manage all these limitations, it is possible to upgrade biogas using microalgae, thanks to their photosynthetic CO₂ reduction capacity. In fact, when microalgae are used for biogas upgrading, the photosynthesis can convert CO₂ present in raw biogas, into biomass and oxygen.

1.2 Microalgae: description and classification

Microalgae, or microphytes, are microscopic algae, typically found in freshwater and marine systems, living in both the water column and sediment (Thurman, H. V. (1997). *Introductory Oceanography*. New Jersey, USA: Prentice Hall College). Microalgae can exist individually, in chains or in groups. They do not have roots, stems or leaves.

Microalgae can perform photosynthesis process: they result really important for life on Earth, because it is estimated that they produce approximately half of the atmospheric oxygen, using at the same time the greenhouse gas carbon dioxide. This last gas is used by microalgae for their photoautotrophic growth (Gustaaf Hallegraeff, 2013).

Because of the different species, microalgae are classified in groups based on different characteristics. First of all, they cover a large range of sizes: from a few micrometres, to a few hundred micrometres. They have a huge morphological variation, such as round, oval, cylindrical, and fusiform cells, as well as projections like thorns, cilia, etc. The biodiversity of microalgae is enormous: it has been estimated that about 200.000-800.000 species exist, but only about 50.000 of these species are actually described (Starckx, Senne - 31 October 2012 - A place in the sun - Algae is the crop of the future, according to researchers in Geel Flanders Today, Retrieved 8 December 2012). Most of these microalgae species produce products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols.

Algae are usually found in damp places or bodies of water; they can be situated both in terrestrial and aquatic environments. Algae require three important components to grow, exactly like plants: light, water and carbon dioxide.

These organisms can be classified in four main types, easily discernible by colour:

- diatoms (*Bacillariophyceae*);
- green algae (*Chlorophyceae*);
- blue-green algae, also called cyanobacteria (*Cyanophyceae*);
- golden algae (*Chrysophyceae*).

In particular, diatoms, green and golden algae are the most diffused ones (Talebi *et al.* 2014; Quinn *et al.* 2014).

Microalgae are primitive organisms, with a simple cellular structure, but they have an interesting property: their surface to volume ratio is normally very large. So, they have the ability to uptake a bigger amount of nutrients (Sheehan *et al.* 1998a, b). The photosynthetic process is similar to the land-plants' one, but, due to the simple cellular structure, they are more efficient in the transformation of solar energy in biomass (Carlsson *et al.* 2007; Chisti 2007). Moreover, another study affirms that phototrophic microalgae can convert solar energy to chemical energy with a greater efficiency (10-50 times) than land plants (Zhou *et al.* "Bio-mitigation of carbon dioxide using microalgal systems: Advances and perspectives").

Carbon, nitrogen, phosphorus and sulphur are the main components of microalgae (Chisti, 2007; Tsai *et al.* 2011; Zeng *et al.* 2011). Obviously, it is impossible to find a general molecular

formula, because of the different species; neither their chemical composition is a constant, because it varies over a large range. However, a minimal nutritional requirement can be estimated using a molecular formula of the microalgal biomass: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti, 2007; Putt *et al.* 2011). Nitrogen is the critical factor for the growth and lipid content regulation of microalgae. Phosphorus, although required in small amounts, must be supplied in excess as it complexes with metal ions (Chisti, 2007). Microalgae can use light and nutrients from their environment, thereby they can optimize the efficiency of utilization for their growth and survival. Moreover, they are extremely resilient microorganism, so, a single species can undergo various metabolism, depending on the available nutrients for growth (Amaro *et al.*, 2011).

1.3 Microalgae: applications

Algae can be used in several sectors, with different applications. In fact, they contain numerous compounds that can be harnessed for commercial use:

Pigments/Carotenoids	B-Carotene, astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, phycoerythrin, fucoxanthin
Polyunsaturated fatty acids (PUFAs)	DHA(C22:6), EPA(C20:5), ARA(C20:4), GAL(C18:3)
Vitamins	A, B1, B6, B12, C, E, biotin, riboflavin, nicotinic acid, pantothenate, folic acid
Antioxidants	Catalases, polyphenols, superoxide dismutase, tocopherols
Other	Antimicrobial, antifungal, antiviral agents, toxins, aminoacids, proteins, sterols, MAAs for light protection.

Table 2: Useful substances in microalgae

The potential of microalgae is high, because of their more efficient utilization of light energy as compared with higher plants. The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* during famine. However, the principal microalgal

technologies began only in the middle of the last century. Nowadays, there are several commercial applications of microalgae.

1.3.1 Microalgae for human food

Microalgae are a rich source of carbohydrates, protein, enzymes and fibres, as well as many vitamins, like A, C, B1, B2, B6, and minerals, like potassium, iron, magnesium and calcium. Because of their composition, they are used like major source of food in many Asian countries, like Japan, China and Korea. Some of the most known algae, used like food, are the green algae *Chlorella Vulgaris*, *Dunaliella Salina* and the cyanobacteria *Spirulina Maxima*. Instead, *Spirulina Platensis* is a blue-green alga, and it is gaining worldwide popularity as a food supplement. It has been shown to be an excellent source of proteins (Colla *et al.*, 2007), polyunsaturated fatty acids (Sajilata, 2008), pigments (Rangel-Yagui *et al.*, 2004; Madhyastha and Vatsala, 2007), vitamins and phenolics (Colla *et al.*, 2007; Ogbonda *et al.*, 2007).

Moreover, some species of microalgae contain big amounts of carotene and beta carotene. The latter one is used like food colouring, food additive and to improve the health and fertility of grain-fed cattle. It was announced recently, by the National Cancer Institute, that Beta Carotene is anticarcinogenic; other studies have found that Beta Carotene is effective in controlling cholesterol and in reducing risks of heart disease. These new findings make Beta Carotene much more valuable and are likely to increase the demand for the product (Commercial and industrial applications of micro algae, Indira Priyadarshani and Biswajit Rath).

1.3.2 Microalgae for cosmetics

Every kind of algae has a typical pigment, normally used to control cell growth and check the trophic level of water. Components of algae are used in cosmetic field like thickening agents, water-binding agents and antioxidants. Microalgae extracts can be mainly found in skin care, sun protection and hair care products. The most successful species are *Arthrospira* and *Chlorella* (Stolz and Obermayer, 2005).

1.3.3 Microalgae and high-value molecules

Microalgae can be a very interesting natural source of new compounds, which can be used as functional ingredients. In fact, some microalgae live in complex habitats submitted to

extreme conditions (for example, changes of salinity, temperature, nutrients, UV-Vis irradiation, etc.), and so they must adapt rapidly to the new environmental conditions to survive. In this way they produce many secondary (biologically active) metabolites, which cannot be found in other organisms.

Moreover, marine microalgae are recognised as an important renewable source of bioactive lipids, and they contain a big amount of polyunsaturated fatty acids (PUFA): it was established that they are effective in preventing or treating several diseases.

1.3.4 Microalgae and biofuels

Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production. Algae offer many advantages:

- They can theoretically produce 1000-4000 gallon/acre/yr: this measure is higher than soybeans and other oil crops;
- Algae do not compete with traditional agriculture, because they are not traditional foods. Moreover, they can be cultivated in large open ponds or in closed photobioreactors located on non-arable land too;
- They can grow in a wide variety of climate and water conditions, and can utilize and sequester CO₂ from many sources;
- Finally, they can be processed into a broad spectrum of products, including biodiesel via trans-esterification, green diesel and gasoline replacements via direct catalytic hydrothermal conversion, and catalytic upgrading, and bioethanol via fermentation, methane via anaerobic digestion, heat via combustion, bio-oil and biochar via thermochemical conversion, and high protein animal feed.

1.3.5 Microalgae in environmental applications

Microalgae have many applications in environmental biotechnology, especially for bioremediation, bioassay and biomonitoring of environmental toxicants. First of all, they can be used for wastewater: its discharge contains several contaminants into the aquatic ecosystems. The high rate algae pond (HRAP) system has been shown to be an efficient system to treat wastewater. This system consists of shallow pond mixed by paddle-wheels to enhance nutrient transfer and photosynthetic efficiency to optimise algal growth.

In addition, immobilised microalgae are useful for enhance the efficiency in the removal of environmental contaminants. In particular, *Chlorella Vulgaris* and *Scenedesmus Obliquus* have been shown to be efficient in removing nitrogen and phosphorus from urban wastewater. Other studies have demonstrated that microalgae can be used as bioassay organisms, to assess the toxicity of pollutants such as heavy metals, pesticides and pharmaceutical (Biotechnological applications of microalgae, Wan-Loy Chu).

1.3.6 Other applications

Often, microalgae are employed in agriculture as biofertilizers and soil conditioners. In fact, the majority of cyanobacteria are capable of fixing atmospheric nitrogen; they also play an important role in maintenance and build-up of soil fertility.

Algal organisms are rich source of biologically active primary and secondary metabolites, that can have a potential interest in the pharmaceutical industry (Rania and Hala, 2008). Many strains of cyanobacteria are known because they produce intracellular and extracellular metabolites, with different biological activities, such as antialgal, antibacterial, antifungal and antiviral activity.

Microalgae are often used as aquaculture feed, in particular for the culture of larvae and juvenile shelland finfish.

1.4 Thesis structure

This work starts with an introduction, containing a brief description of microalgae and all the possible application of microalgae. The second chapter contains information about the state of the art. In the first part LED illumination field is treated, while in the second part different types and forms of photobioreactors are listed. The third chapter is about methods and materials used during experiments. It contains a brief description of all instruments and machinery used. In Chapter 4 all the results are reported, in form of graphics, tables and formulas. The thesis ends with Chapter 5, containing the discussion of results and conclusions of the entire work.

2. STATE OF THE ART

This chapter is divided into two parts: the first one is about state of the art of LED illumination. So, it contains a brief introduction of this kind of light source and its working modes, and all the information about its effects on algal growth. The second part treats state of the art of photobioreactors: it contains a description of all the major forms of these reactors. The chapter ends with a list of study cases realized in the past with photobioreactors.

2.1 LED illumination

Microalgae are photoautotrophic organisms, so they can use light as their only energy source in photoautotrophic conditions. This means that they can produce complex organic compounds from simple substances, using light as energy source. So, because microalgae cells absorb light photons as nutrients, then wavelength, light intensity and all the other light characteristics are fundamental parameters in microalgal growth. In this study, LED illumination was used, because it has specific narrow bands which can produce cost effective irradiance, and this is the ideal spectral for microalgal growth.

2.1.1 LED description

LEDs, light-emitting diodes, are solid-state semiconductors and they can produce light with a fine emission peak. They consist of a positive layer and a negative layer. Depending on the materials of these layers, electrons have different energies, and so different wavelengths. Because of these characteristics, distinct fragments of photosynthetically active region (PAR, that is the spectral range of solar radiation, generally between 400 and 700 nm, that photosynthetic organisms can use during the process of photosynthesis) can be studied in independent way. Thus, every specific photosynthetic response of different organisms can be analysed: in fact, the main absorption peak in the PAR depends on the particular chlorophyll and carotenoid contents of the specific specie examined (Shulze *et al.*, 2014).

The positive layer has an excess of electron holes in the valence band, while the negative one has an excess of electrons in the conduction band. When a suitable voltage is applied, electrons are able to recombine with electron holes; in this way energy is produced in the form of photons, which are released between these two semiconductors layers. Generally, the material used in LEDs is Aluminium-Gallium-Arsenide (AlGaAs). Depending on the different

wavelengths involved, LEDs produce several colour lights. So, light emitted from this device depends on the type of semiconductor material used for the layers. For example, using Gallium-Arsenide, it is possible to produce infrared light. Using Gallium and Phosphorus produces red or green light, while Gallium-Arsenide-Phosphorus give a red or yellow light, and so on.

LEDs generally need low voltage and current. Moreover, the total power output is normally lower than other light sources. Finally, the time response is very low too, generally about 10 ns.

2.1.2 Comparison with other artificial light sources

Photoautotrophic growth of microalgae needs several important elements, such as nutrients (like nitrogen and phosphorus), CO₂ and a light source. Light source can be natural, from solar radiation, or artificial. Artificial light can provide a better regulation of the photosynthetic photon flux density (PPFD), photoperiod and light spectra in microalga production. An important type of artificial light, mostly used in the past, is the fluorescence lamp (FL). These lamps have a wider emission spectrum than LEDs have. Thus, LEDs are long-lasting (about 50000 hours), mercury-free and fast responding (nanosecond scale). Hence, LEDs can be used to adjust the biochemical composition of biomass. LEDs have dimensions of several millimetres, so they can be assembled to form many lamp configurations, such as panels, strips, cylinders or small spheres (Heining *et al.* 2014). For this reason, LEDs result more flexible than fluorescent lamps in the photobioreactor illumination field. Furthermore, they need a less complex equipment to generate collimated light, then FLs, which requires instead larger size and does not allow downscaling of the illuminated area. When electric current is converted to light in LEDs and FLs, energy losses occur, due to thermal dissipation and inward light reflection and reabsorption. This obviously constitutes a problem, because it is a loss of efficiency. Reduction of this inefficiency has resulted in higher power conversion efficiency (PCE) for LEDs (up to 50%) compared to gas-discharge lighting technologies, such as FLs (about 30%). Up until now, primarily LEDs and fluorescence lamps were used as artificial light sources.

An alternative and innovative way of PAR generation could be organic light emitting diodes, that can emit monochromatic light, or they can be multilayer devices containing red, green and blue organic emitters. Its emission spectrum, light extraction efficiency (various recent studies demonstrate that it can arrive until 80%) and intensity of emitted light depend on the applied voltage and the composition of the organic layers (Krujatz *et al.*, 2016).

2.1.3 Effects of light on algal growth

As mentioned above, algae need a light source for their growth. However, every specie of microalgae needs a different type of light, according, mainly, on its pigments content. To maximize photosynthetic efficiency, every photon emitted from a light source should be captured by the photosynthetic apparatus of microalga. In order to achieve this condition, light source should have a complete spectral matching with the photosynthetically active spectrum (PAS). So, in order to choose the correct light for the culture, consecutive steps must be followed:

- 1) find the major pigments for the algae strain;
- 2) choose a light that emits radiation in correct range to be absorbed by the pigments in the algae;
- 3) set up this light next to the tank.

Microalgae can absorb, from the PAR, different wavelengths of light, depending on the types of pigments that they contain. Some of the most important pigments are:

- *Chlorophyll-a*- All plants, algae, and bacteria;
- *Chlorophyll-b*- Chlorophytes and land plants;
- *Carotenoids*- In all plants and algae;
- *Phycoerythrin*- Red algae (Porphyridium, Rhodomonas);
- *Phycocyanin*- Cyanobacteria (spirulina, Arthrospira).

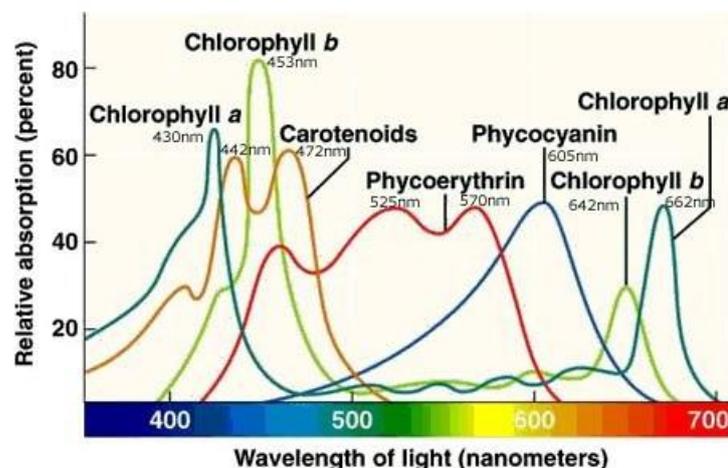


Figure 1: Pigments absorption spectrum (algaeresearchsupply.com)

The last diagram explains how a coloured light is absorbed by a particular pigment: for example, green light is generally not absorbed by *Chlorophyll a* and *b* and *carotenoids*, whereas it is absorbed by *Phycoerythrin*, the red pigment (red is the complementary colour of green). So, for the growth of green microalgae, green lamps are not advised.

Photosynthetic organisms, like microalgae, can use, in the process of photosynthesis, a spectral range (wave band) of radiation from 400 to 700 nm (PAR), which corresponds to the visible spectrum. In fact, light sources with a wavelength higher than 750 nm have a too low energy content to mediate chemical change; energy absorbed in this wave band can only be converted to heat. Further, wavelength radiation below 380 nm have such a high energy content that it ionizes the molecules and consequently can be quite destructive to living biological systems. So, PAR is a measure of the energy of solar radiation intercepted by *chlorophyll a* and *b*, and it is concentrated in red and blue bands, with maximum peaks at 430 and 680 nm. The number of photons at blue or red wavelength that can be captured by a molecule of chlorophyll in microalgae depends on the cellular architecture, pigment conformation and chloroplast arrangement. In the recent years, Keeling affirmed that microalgae prefer red (660 nm) or blue (420-470 nm) light to grow. In fact, red light is fundamental for the growth of chlorophytes, so, photons with wavelengths of 660-680 nm produce the highest quantum efficiency in most plants and algae which contain *chlorophyll a* and/or *b*. Then, efficiency decreases when wavelengths become higher. Kim *et al.* found that changing the ratio between red and blue light affected nutrient utilization more than biomass production: this is due to increased enzyme activity. Furthermore, Mohsenpour and Willoughby found that also green light is fundamental, because it can induce pigmentation in algae.

Regarding white light LEDs, it is important to say that true white-light-emitting LEDs are not available. In fact, this kind of device is difficult to build because LEDs typically emit one wavelength, whereas white does not appear in the spectrum of colours; instead, to obtain white colour, a mixture of wavelengths is needed. So, in order to create white LEDs a simple trick is used: several coloured LEDs are mixed to create a spectral power distribution that is perceived by the eye as white.

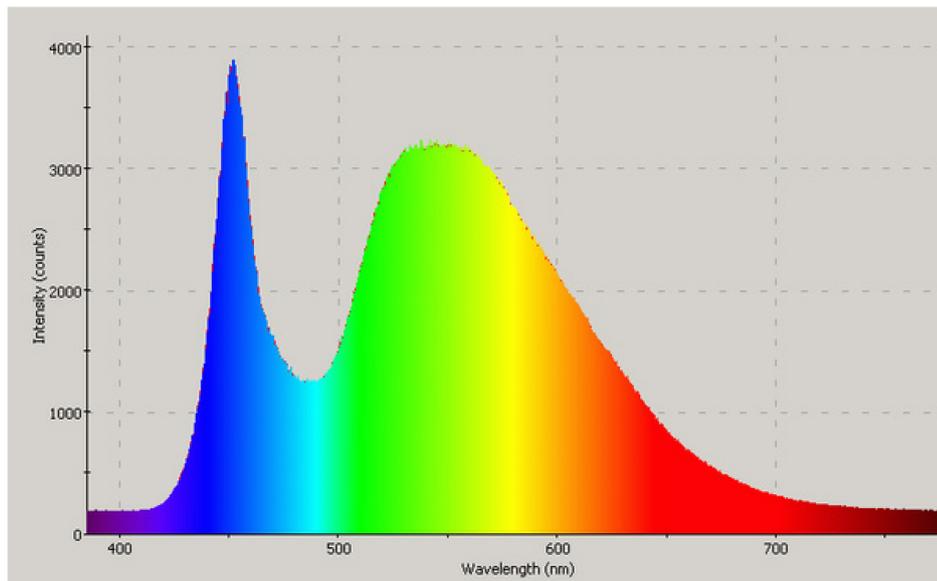


Figure 2: Typical white led spectrum (algaeresearchsupply.com)

While blue and red lights are suboptimal for mass cultivation of microalgae, warm white and yellow lights are fundamental to obtain highest areal productivity in culture growth. Yan *et al.* realized an experiment for the photosynthetic CO₂ uptake by microalgae for biogas upgrading, using various light wavelength and intensities. They used three different monochromatic LEDs, red, blue and white respectively. As a result, white light wavelength was better than other two monochromatic LEDs for biogas upgrading, because it achieved significantly higher microalgae growth dry weight and removal efficiency of biogas CO₂ than the rest of the monochrome LED light wavelengths treatments. In fact, white spectrum covered the ones of blue and red, so it showed a mixed effect of the complete light spectrum on microalgae.

2.2 Algae cultivation systems

Microalgae can be cultivated in open, inexpensive, systems, like natural ponds, circular ponds with a rotating arm, raceway ponds and lakes, or in closed systems, generally called photobioreactor (PBR).

2.2.1 Open ponds

Open ponds can be categorized into two groups: natural waters, such as lakes and lagoons, and artificial ponds. Usually, algae grow in cultivation called “raceway ponds”, in which algae, water and nutrients circulate around a racetrack. Paddlewheels provide the flow, while algae remain suspended in the water and they are circulated back on a regular frequency. These ponds are usually shallow, because algae need to be exposed to sunlight, which can penetrate the pond water only to a limited depth. The systems usually work in a continuous mode, and the feed is added in front of paddlewheel.

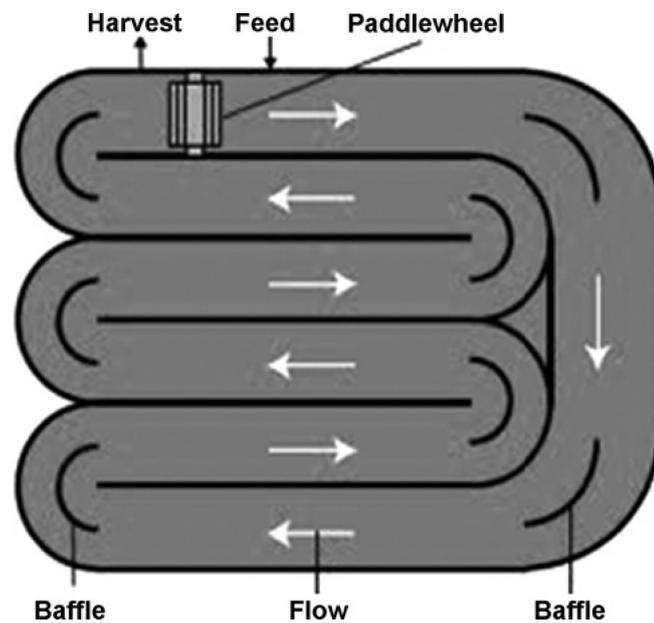


Figure 3: Schematic raceway ponds



Figure 4: Picture of open system raceway (Source: Mission 2014)

The major advantages of open ponds are their cheapness and simplicity: they require only a trench or pond, and they have the largest production capacities relative to other systems of comparable costs. For these reasons, open ponds present low production costs and low operating costs.

On the other hand, open systems do not offer control on temperature and lighting, so, for example, growing season depends on location and often it is limited to warmer months. However, the biggest disadvantages include uneven light intensity, evaporative losses, diffusion of CO₂ to the atmosphere, and they also need large areas. Furthermore, many predators can contaminate cultivations. Consequently, commercial production of algae in open culture systems is reserved to only those organisms that can survive and grow under extreme conditions.

In order to solve these problems, researchers have tried for closed ponds. Here, environments are kept under control. Their cost is higher than open ponds, but they are less expensive than photobioreactors. Closed ponds consist in coverage the pond with a translucent or transparent barrier, using materials like plexiglass.

2.2.2 Photobioreactors

Photobioreactor is a closed and illuminated culture container, realized for controlled biomass production. So, these systems have not direct exchange of gases or contaminants with external atmosphere. They have high cost, but many advantages too:

- They minimize contamination, and allow axenic cultivation of algal monoculture;
- Photobioreactors better control key parameters, such as pH, temperature, light, CO₂ concentrations and so on;

- They offer less CO₂ losses, and prevent water evaporation;
- PBRs permit higher cell concentration than open systems.

Moreover, closed PBRs also provide reproducible cultivation conditions, a good heat transfer control, a better flexible technical design and, finally, a better product quality. Closed PBRs have a productivity limited by various design features, but, regardless of different designs, PBRs need to work under suitable illumination conditions, with an optimised surface area to volume ratio, adequate mass transfer properties and a correct dark/light cycle. Furthermore, every PBR type is mainly composed by four phases: solid algal cells, a liquid growth medium, gaseous H₂ product and the superimposed light radiation field.

The critical parameters of all types of PBRs are light intensity, wavelength, temperature, agitation and mixing, and nutrient delivery and dilution. A collection system is required, in order to measure H₂ production, the algal growth, the dissolved oxygen concentration (it is necessary to understand if the ambient is anaerobic) and the uptake rates of fundamental nutrients.

Below, several designs of photobioreactors realized and developed for algal production are described.

Vertical tubular PBR

It is constituted by a vertical transparent tubing, in order to allow light penetration. At the bottom of the reactor an air sparge system is placed: it transforms sparged gas into tiny bubbles. This system allows overall mixing, mass transfer of CO₂ and it also removes O₂ produced during photosynthesis. Vertical tubular photobioreactors can be divided into two main groups: bubble column and airlift reactor, according to the specific mode of liquid flow.

The first one is a cylindrical device, with height greater than twice the diameter. Its capital cost is low, and it has high surface area to volume ratio, lack of moving parts, good heat and mass transfer, efficient release of O₂ and residual gas mixing. Photosynthesis efficiency depends on gas flow rate, that depends, in turn, on the light/dark cycles.

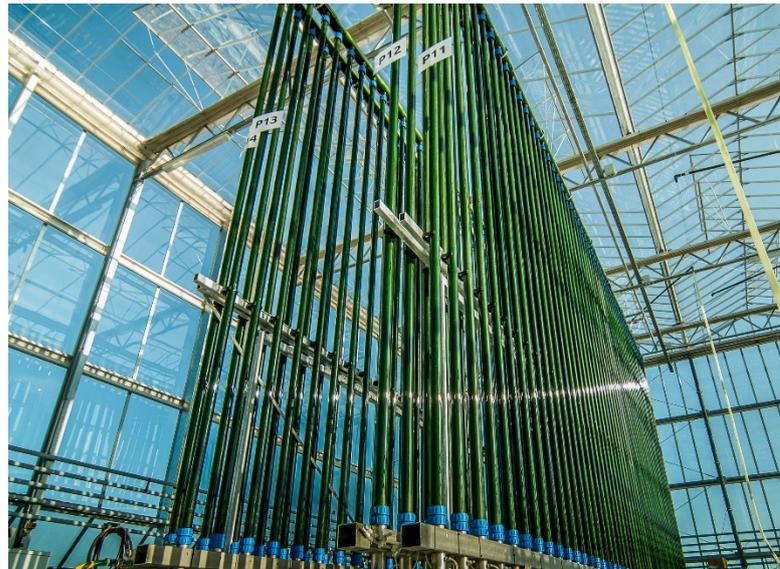


Figure 5: Ecoduna's world-patented vertical photobioreactor constructed with glass tubes (Source: schott.com)

Air lift reactors are realized with two interconnecting zones. One of the tubes is used for gas mixture sparging, and it is called *riser*. The other region does not receive the gas, and it is called *downcomer*. These systems can exist into two main forms: external or internal loops. In the first one, riser and downcomer are physically separated by two different tubes, while in the internal one these regions are separated by a split-cylinder or a draft tube.

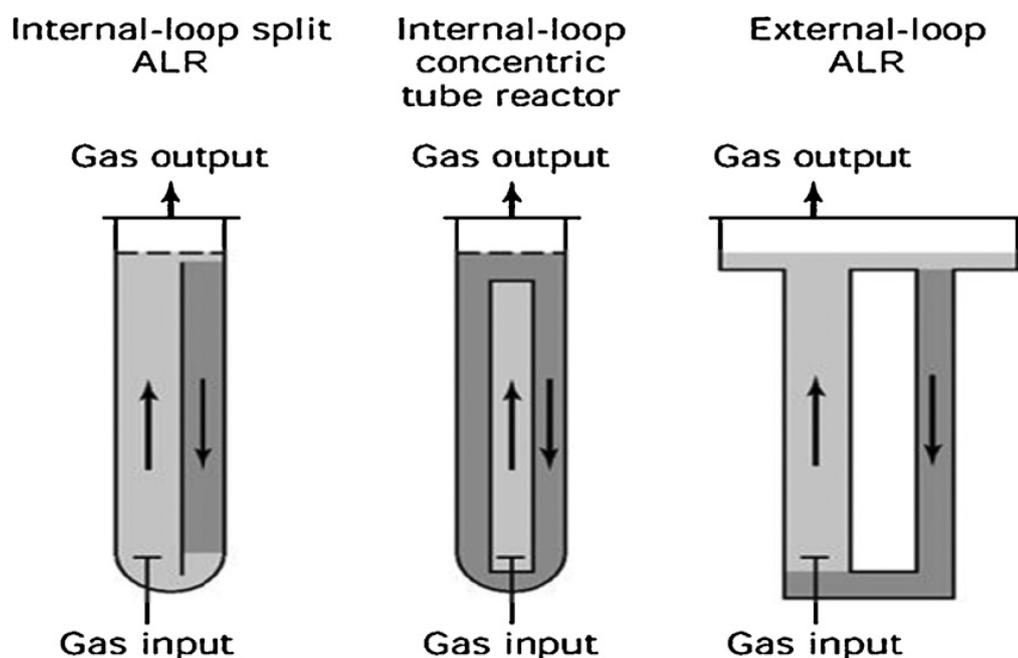


Figure 6: Different types of air lift PBR

Within this category of PBR, rectangular airlift photobioreactors are proposed too. Their main advantages are the optimal mixing conditions and the high photosynthetic efficiency. On the other hand, they are very complex, and it is difficult their scale-up.

Flat panel PBR

The flat panel is a cuboidal reactor, usually made by glass, polycarbonate, plexiglass, etc. The most important characteristic of this device is its surface area to volume ratio, that is the highest among all PBRs. This property leads to the best photosynthetic efficiencies observed for any PBR. Normally, flat panel reactors are vertical, with the light source incident on the reactor from one side. The region immediately adjacent to reactor illuminated surface is a photic zone: here, light saturation, and so photoinhibition of algal growth and H₂ production processes, often occurs. Moreover, increasing the distance from this photic zone, energy available to algal cells rapidly decreases. Flat panel reactors are subject to moderately low mass transfer rates, because the light path, that is the space between panels, is very limited, and this characteristic decreases the clearance efficiency of the dissolved O₂ produced by photosynthesis. In order to obtain an optimal algal growth, a good O₂ diffusion rate through the reactor is necessary. Agitation system is provided by bubbling air, introduced through perforated tube or by rotating it through a mechanical motor.

On the other hand, flat panel reactors have some disadvantages, such as the scaling-up requirements (many compartments and support materials are necessary), the difficulty in controlling system temperature, the possibility of algal cells fouling on the internal wall of reactors, and, finally, the incompatibility with some algal species. An example of these photobioreactors was built up by Barbosa *et al*, made of polycarbonate and with a surface area to volume ratio of 0.34 cm⁻². It was illuminated by 10 fluorescent tubes, with a total light intensity of 1000 μmol photons m⁻²s⁻¹. This model was modified by Zhang, who introduced baffles for the improvement of mixing and agitation. Other engineering modification were added by Iqbal, who improved, in this way, mixing rate, while escape corners were eliminated. Tredici and Zittelli realized a flat horizontal panel divided into five channels, with two plexiglass manifolds at the top and at the bottom. Surface area to volume ratio became 40 m². During the cultivation of *Chlorella Sorokiniana* a flat panel with short path length was used: submitting these algae to high irradiance conditions, volumetric productivity obtained was 12.2 g L⁻¹d⁻¹.

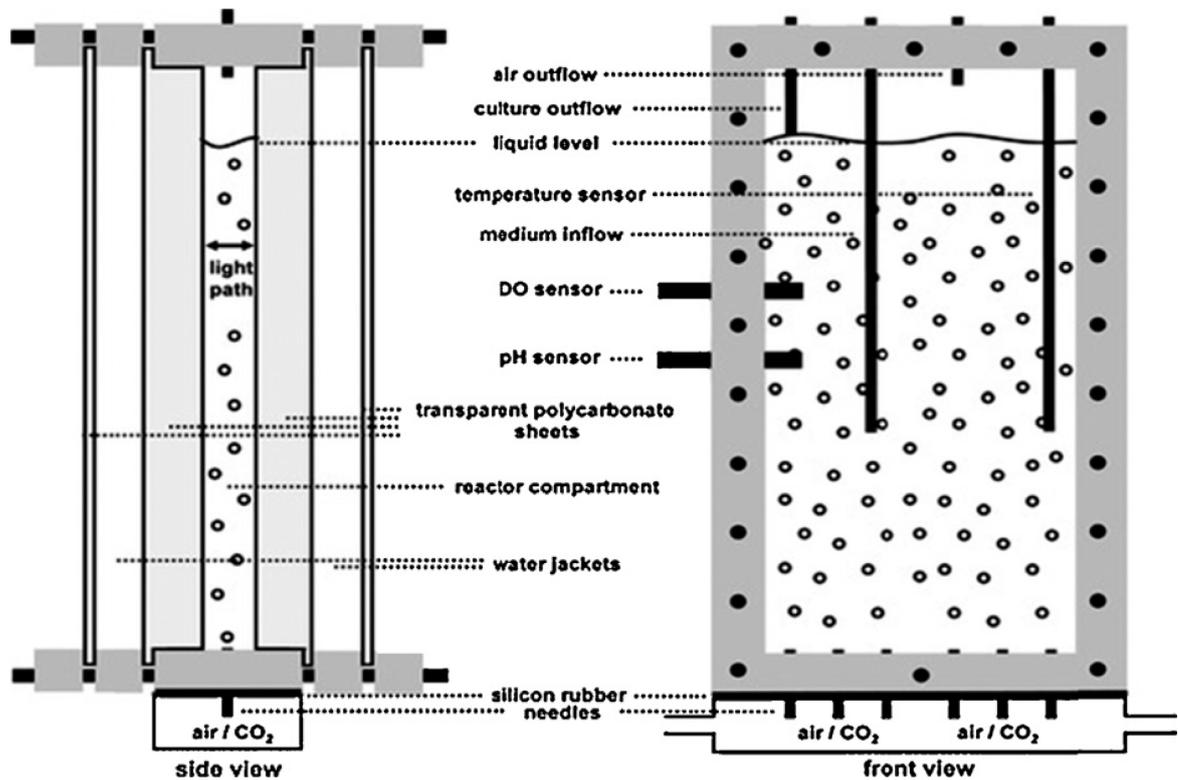


Figure 7: Front and side view of the flat PBR

Another example is the study of H₂ production, done using the green alga *Chlamydomonas reinhardtii*, realized by Tamburic *et al.* In fact, algae have the capacity to harness sunlight to photosynthetically produce molecular hydrogen from water. This process is totally carbon neutral, and it also allows carbon dioxide sequestering and biomass accumulating during algal growth. During this study, a one-litre vertical flat plan PBR was used. Non-heating illumination was provided by a panel of LEDs, while reactor body was realized by two compartments made by transparent polymethyl methacrylate sheets:

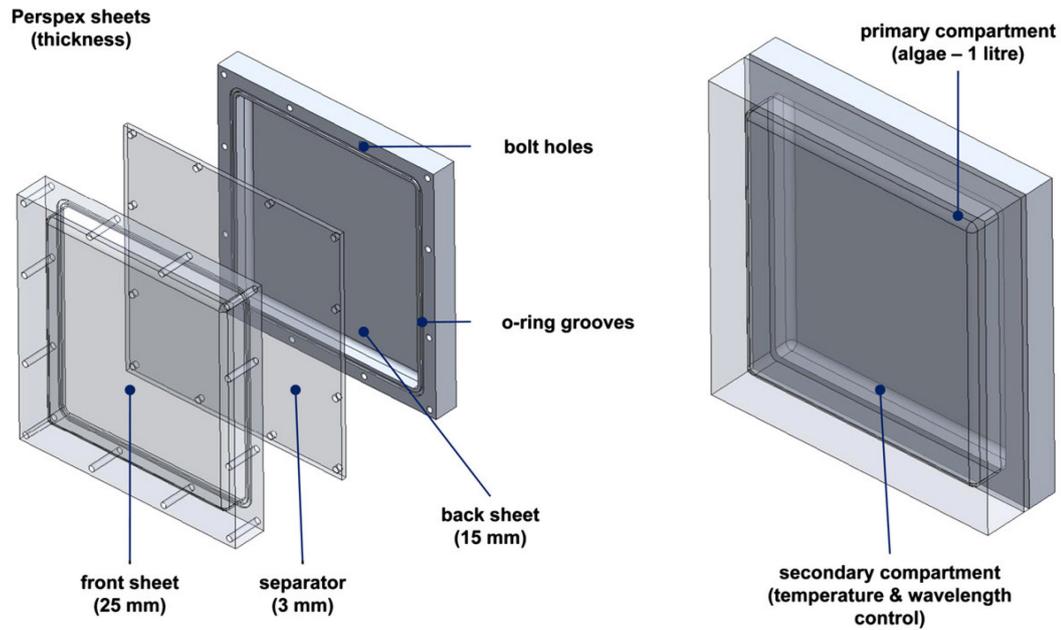


Figure 8: Novel dual-compartment vertical flat-plate reactor design (left: exploded view; right: assembled view)

Horizontal tubular photobioreactor

They consist in horizontal reactors with several forms, from the tubular to the loop shape one, from the α -shape to the parallel set of tubes. Its shape assumes a fundamental role, because it establishes its orientation towards sunlight. For this reason, every form of horizontal PBR has a different light conversion efficiency. However, photosynthetic efficiency and volumetric productivity are normally higher than those of flat panel bioreactor. On the other hand, their biggest disadvantage is the very high energy consumption: it has a value of 2000 Wm^{-3} , while bubble column and flat panel photobioreactors have value of about 50 Wm^{-3} . They need high energy input because it is necessary to reach high linear velocities, in order to achieve turbulent conditions with sufficient short light/dark cycle.

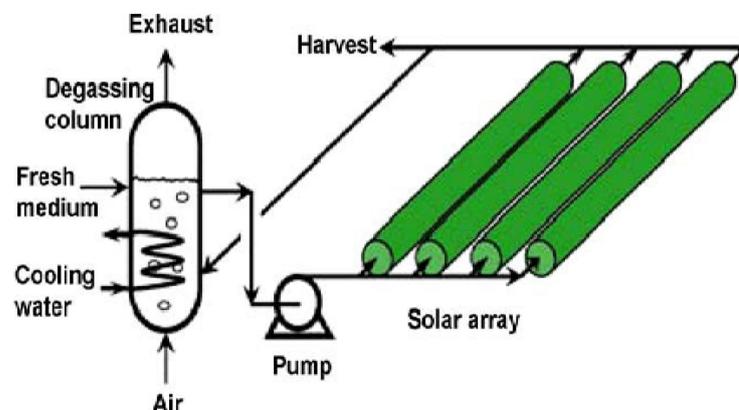


Figure 9: Working of horizontal tubular PBR



Figure 10: Horizontal tubular PBR (Picture source: algarefinery.com)

Helical PBR

Helical photobioreactor is realized with a coiled and transparent small tube, which is flexible too, and a degassing system, that can be separated or attached. Culture must arrive to the degassing unit, through the tube; to do this, a centrifugal pump is used. Several studies were conducted within this field, using different algal species. Carbon dioxide and algal volume can be injected from the top to the bottom, or, vice versa, from the bottom to the top: with last method photosynthetic efficiency is higher than the first one.

The scale-up of these systems can be done simply adding light harvesting unit, but the big amount of energy required by centrifugal pump makes this device really expensive, and so it does not cover an important role on the commercial market.

Morita, Watanabe and Saiki gave a conical shape to the helical PBR, and their study showed that a conical angle of 60° increased photo-receiving area and photosynthetic productivity by a factor of two. (Morita *et al.*, Investigation of photobioreactor design for enhancing the photosynthetic productivity of microalgae, 2000). Cone shape has the big advantage of light harvesting efficiency, with the same shape and occupied area of the helical one. Moreover, they need less energy and present fewer mechanical stresses imposed to algal cells. The scale-up of these systems is obtained increasing the number of light harvesting units, but in this way, obviously, energy losses increase too, because of the complicated path that the flow must take.

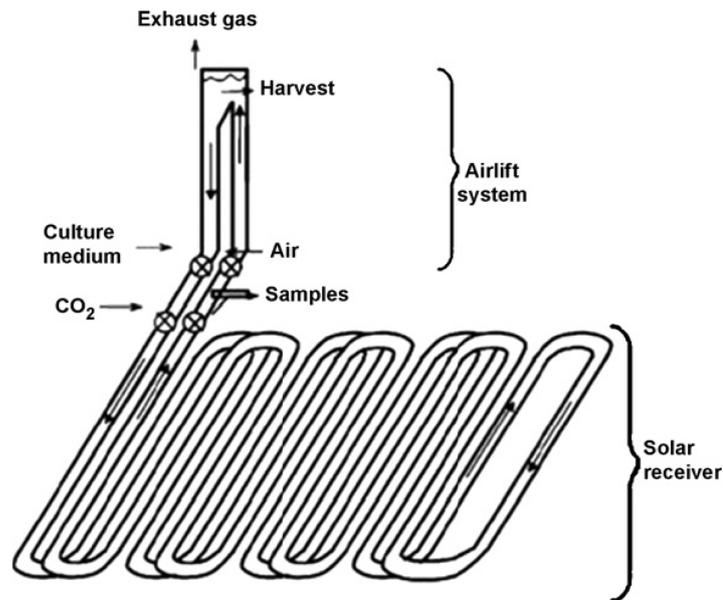


Figure 11: Helical PBR

Stirred tank PBR

Stirred tank reactors are the most popular kind of reactors. They are transformed in photobioreactors simply adding, externally, fluorescent lamps or optical fibres. Impeller, with different shapes and dimensions, is necessary, in order to provide mechanical agitation, while baffles are used in order to reduce vortex formation. Air, enriched with CO₂, is bubbled from the bottom of the reactor, and it constitutes the source of carbon needed by microalgae for their growth. The biggest disadvantage of this system is its low surface area to volume ratio, that means low light harvesting efficiency. In order to solve this problem, optical fibres were used too, but they had another disadvantage, that is hindrance in the mixing pattern.

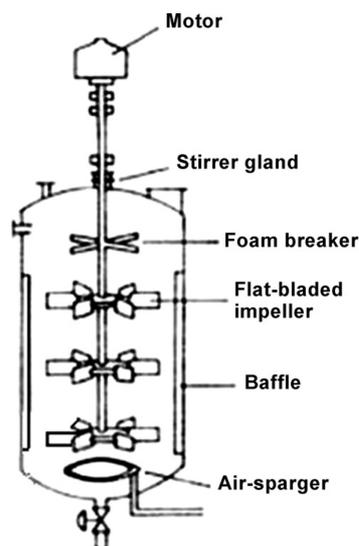


Figure 12: Stirred tank PBR

Final considerations about PBRs

In order to take advantages of one kind of PBR, and overcome disadvantages of another one, hybrid type PBRs were realized. One example is given by Fernandez *et al.*, who used integrated airlift system and external tubular loop placed horizontally in a thermostatic pond of water. While external loop controls the temperature of the culture and gives high surface area to volume ratio, airlift system is used as degassing unit, where other probes can be introduced, in order to control other process parameters too. In this way, culture variables could be better controlled, while productivities became higher and power consumption decreased (Fernandez FGA, Sevilla JMF, Perez JAS, Grima EM, Chisti Y. Airlift driven external loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance).

Key parameters of photobioreactor are light distribution, mass transfer, shear stress, scalability and biology of algae cells. At the moment, none of the single bioreactor mentioned satisfies all these requirements. Hybrid reactors seem to be a good option, because they demonstrate to be useful in mass production of algae, as compared to single bioreactors. In the future, several studies may be made to combine different kinds and shapes of bioreactors, in order to realize appropriate bioreactors for mass algal culture.

Culture systems	Prospects	Limitations
Open ponds	Relatively economical, easy to clean up after cultivation, good for mass cultivation of algae	Little control of culture conditions, difficulty in growing algal cultures for long periods, poor productivity, occupy large land mass, limited to few strains of algae, cultures are easily contaminated
Vertical-column photobioreactors	High mass transfer, good mixing with low shear stress, low energy consumption, high potentials for scalability, easy to sterilize, readily tempered, good for immobilization of algae, reduced photoinhibition and photo-oxidation	Small illumination surface area, their construction require sophisticated materials, shear stress to algal cultures, decrease of illumination surface area upon scale-up
Flat-plate photobioreactors	Large illumination surface area, suitable for outdoor cultures, good for immobilization of algae, good light path, good biomass productivities, relatively cheap, easy to clean up, readily tempered, low oxygen build-up	Scale-up require many compartments and support materials, difficulty in controlling culture temperature, some degree of wall growth, possibility of hydrodynamic stress to some algal strains
Tubular photobioreactors	Large illumination surface area, suitable for outdoor cultures, fairly good biomass productivities, relatively cheap	Gradients of pH, dissolved oxygen and CO ₂ along the tubes, fouling, some degree of wall growth, requires large land space

Figure 13: Advantages and limitation of the principal PBR types

2.3 LED technologies applied to commercial algae cultivation

In the past, public and verifiable information about applications of LEDs in microalgae cultivation was scarce. However, at the moment, thanks to the increasing interest in microalgal research, a few cultivation systems have become commercially available. Some examples are reported below.

- Labfors 5 Lux Flat Panel photobioreactor (INFORS HT, Bottmingen, Switzerland) has a total working volume of 1.8 L. It is created as a narrow flat panel airlift photobioreactor, with an optical light path of 20 mm. This reactor has an automatic temperature-control system, and it is illuminated from one side by a LED panel, with 260 high-power LEDs having a warm-white emission spectrum. Moreover, it presents a light intensity of up to $2400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Apel and Weuster-Botz 2015). In particular, this PBR system can be used to improve process parameters, such as the triacylglycerol accumulation in *Scenedesmus obliquus* (Breuer *et al.* 2014).
- The photobioreactor FMT 150 series (PSI, Brno, Czech Republic) is available with a flat-vessel volume of 0.4, 1.0, and 3.0 L. It enables tight process control achieved by multiple sensors too, for example temperature, pH, O₂, and CO₂. The optical density (OD) is recorded in real time at 680 and 720 nm by two far red LEDs and a photodiode. The illumination, in the standard version, is realized by either a white-red LED panel or a blue-red LED panel, with emission peaks at 450 and 620 nm, respectively. The maximal light intensity is $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, but an upgraded version provides intensities up to $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Nedbal *et al.* 2008).
- PSI offers the Multi-Cultivator MC 1000, which consists of eight cultivation tubes with 85 mL culture volume each. This LED presents a warm-white or cool-white illumination too, that can be adjusted individually for each tube. It can reach photon flux densities of up to $900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. A water bath surrounds all the eight tubes; its aim is to control the temperature, maintaining it constant.
- Phenometrics (Lansing, USA) offers PBR101, created for the simulation of environmental conditions and optimization of open ponds. In this case, the working volume is 0.45 L, while illumination is obtained by a single high-power white LED, placed in the top, with a spectrum comparable to sunlight. Its intensities exceed $3000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

3. METHODS AND MATERIALS

This chapter is aimed at giving information about all the procedures, instruments, equipment and materials used during the execution of experiment.

3.1 Microalgae and nutrients

The selected strain used for this study is *Scenedesmus Obliquus* (SAG 276-3a), that is a green microalgae species of the genus *Scenedesmus*, notable for the genetic coding of its mitochondria, whose habitat is typically the freshwater. They have a typical shape, similar to an eye. This microalga was selected on the basis of precedent studies in this context (Franchino *et al.*, 2013).

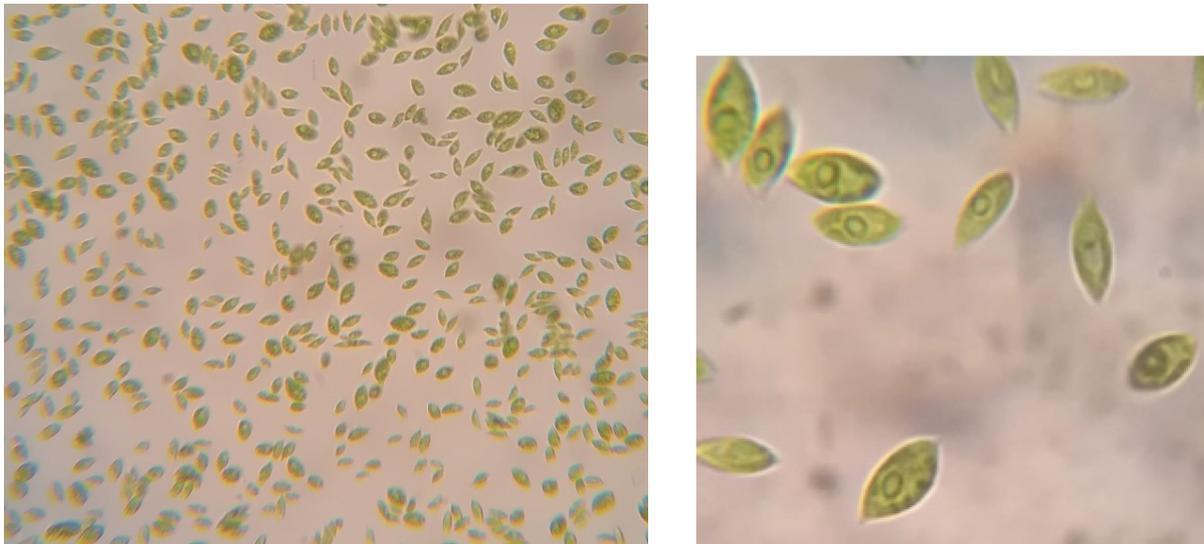


Figure 14: a) On the left, view under a microscope with 100X; b) On the right, view under 1000X magnification

In literature, *Scenedesmus Obliquus* was already used in several studies, with different aims. The main titles and results are reported below:

- “*Scenedesmus obliquus* CNW-N as a potential candidate for CO₂ mitigation and biodiesel production”, by Ho *et al.* *Scenedesmus obliquus* was cultivated via a two-stage system with 10% CO₂, to mitigate CO₂ emissions and simultaneously produced biodiesel. As a result, the biomass productivity was 292.50 mg L⁻¹ d⁻¹, lipid productivity 78.73 mg L⁻¹ d⁻¹, while CO₂ consumption rate was 549.90 mg L⁻¹ d⁻¹.

- “*CO₂ biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO₂ levels*”, by Tang *et al.* In this study, Scenedesmus obliquus and Chlorella pyrenoidosa were cultivated with 0.03%, 5%, 10%, 20%, 30%, 50% CO₂. These microalgae could grow at 50% CO₂ (>0.69 g L⁻¹), while grew well (>1.22 g L⁻¹) under CO₂ concentrations ranging from 5% to 20%. However, these microalgae strains had best growth potential at 10% CO₂.
- “*Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor*”, by De Morais and Costa. During this experiment Scenedesmus obliquus and Spirulina were cultivated at 30°C in a temperature-controlled three-stage serial tubular photobioreactor. The resistance of these organisms to limitation and excess of carbon dioxide was calculated too. Finally, this study achieved the capacity of the system to fix this greenhouse gas.
- “*Photobioreactor strategies for improving the CO₂ fixation efficiency of indigenous Scenedesmus obliquus CNW-N: Statistical optimization of CO₂ feeding, illumination, and operation mode*”, by Ho *et al.* Statistical experimental design and bioreactor strategies were applied to improve CO₂ fixation ability of Scenedesmus obliquus. Under the optimal conditions, the highest biomass productivity (1030.2 mg L⁻¹ d⁻¹), photosynthesis efficiency (10.45%), and CO₂ fixation rate (1782.3 mg L⁻¹ d⁻¹), gained during this study, were higher than almost all the values reported in other studies.

Microalgae use nutrients for their growth. In particular, during this experiment, it was used BG-11 medium for their cultivation. BG-11 medium is a solution of distilled water and different chemicals, used like nutrients by microalgae. The following *Table 3* reports BG-11 medium composition:

Compound	Concentration (g/l)	Stock Solution	
NaNO₃ (Sodium Nitrate)	1.5	200X	} Solution 1
K₂HPO₄ (Dipotassium Hydrogen Phosphate)	0.04	1000X	
MgSO₄ · 7H₂O (Magnesium Sulfate Heptahydrate)	0.075	1000X	} Solution 2
CaCl₂ (Calcium Chloride)	0.036	1000X	
C₆H₈O₇ (Citric Acid)	0.006	1000X	
Na₂CO₃ (Sodium carbonate)	0.02	1000X	

$C_6H_{11}FeNO_7$ (Ferric Ammonium Citrate)	0.006	1000X	Solution 3
$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (Na_2EDTA)	0.001	1000X	
H_3BO_3 (Boric Acid)	2.86×10^{-3}	1000X	Solution 4
$MnCl_2 \cdot 4H_2O$ (Manganese Chloride Tetrahydrate)	1.81×10^{-3}		
$ZnSO_4 \cdot 7H_2O$ (Zinc Sulfate Heptahydrate)	0.222×10^{-3}		
$MoNa_2O_4 \cdot 4H_2O$ (Molibdenum Sodium Oxide)	0.39×10^{-3}		
$CuSO_4 \cdot 5H_2O$ (Copper Sulfate Pentahydrate)	0.079×10^{-3}		
$Co(NO_3)_2 \cdot 6H_2O$ (Cobalt Nitrate Hexahydrate)	0.049×10^{-3}		

Table 3: BG-11 medium composition

Before reaching photobioreactor, microalgae must undergo a long growth period, which can last many weeks. They arrive in laboratory like slant cultures, and they are subjected to light, to a temperature of 20-25°C, until the entire surface of agar (on which they are disposed) is fully covered by microorganisms. At that point, algae are inoculated inside 400 ml glass bottle, containing 100 ml of BG-11 medium. This bottle is fixed to an orbital shaker with 150 rpm rotation speed, and it is illuminated by lamps, as visible in the picture below.



Figure 15: Orbital shaker

After two weeks, volume is doubled, and an air sparging system is added. Then, microalgae go to a centrifugation process, with 4000 rpm for five minutes. The product of this phase is newly inoculated, inside six bottles. In this way, the total volume is six-times higher

than the first one, and it is high enough to be inoculated inside the column, after the necessary period of growth.

The column, a tube made of polycarbonate, is the last phase before the introduction into the photobioreactor. This column is illuminated too, and it permits further algae's growth. Carbon dioxide is introduced inside column, both in form of air and in pure form. Only five of the six bottles are inoculated inside column, while the other one is used for others centrifugation processes.

After an opportune period of growth, microalgae can be transferred into the flat panel.

3.2 Photobioreactor system

The system in which microalgae grow is composed principally by two parts: a planar photobioreactor and a tank. Microalgae's growth occurs in the first one, while tank is necessary for mixing microalgal volume with nutrients and to wash dissolved oxygen produced during microalgae photosynthesis, and for control all the process variables.

3.2.1 Description of the system

The circulation of liquids under pressure inside the panel is allowed by a system, object of a patent, that allows to realize a hydraulic circuit inside the honeycombed panel. This system generates a hydraulic seal with the internal sectors of the panel, which can be used to generate a serpentine hydraulic circuit.

To guarantee the tightness between the two parts of the sealing and connecting mechanism, the two parts are coupled to each other through a particular geometry. Then, these parts are assembled in the panel, thus connecting the two channels of the panel. All these details were realized with plastic polymers, having a transparent matrix.

The main apparatuses are:

- ***Apparatus for exposure of culture liquids to light***

It is mainly constituted by a transparent plastic honeycomb panel and sealing systems. During their passage in this apparatus, microalgae follow the paths obtained with the use of the sealing and connection systems between an alveolar element and the following one. While they pass, they are irradiated by the light coming from the outside, absorbing the energy necessary to carry out the internal chemical reactions, indispensable to the assimilation of the carbon

dioxide and its subsequent transformation into other types of molecules. Because of the possibility of providing circuits composed of a considerable number of channels, microalgae can be exposed to light for a significant amount of time. Consequently, the amount of light they can receive for each complete cycle of the system is maximized.

- ***Liquid handling device***

The movement of the liquid is necessary to ensure the entire population of microalgae the same exposure to the light, a constant accessibility to the nutrients and a good mixing of liquids: so, the parameters should have a range of gradients the most reduced possible, within the whole system. Furthermore, the correct circulation speed of the culture liquids, within the system, reduces the risk of superficial adhesion of the organisms to the walls of the panel (fouling) and reduces the risk of formation of agglomerations of various kinds. In fact, fouling involves a reduction in the amount of light available for microalgae, and requires subsequent mechanical and/or chemical cleaning of the channels; these consequences can be considerably reduced choosing a correct speed of the liquid, according to the microalgae growth.

The pumps for moving liquids are chosen according to the amount of liquid that must be moved, but the choice must be made in order not to damage the cells too, when the speed imposed on the liquid becomes relevant. So, the choice of the pump is not based only on its mechanical or volumetric performance, but also considers that the liquid power transfer system does not cause excessive damage to the cells. The correct choice of liquid handling device also allows to reduce energy consumption related to the circulation of liquids inside the system.

- ***Nutrient administration apparatus***

This apparatus is fundamental to provide the organisms with all the substances necessary for their physiological needs. So, it is necessary when cultivation systems require periodic or occasional reintegration of substances essential for the growth and maintenance of cells. It is simply constituted by a tank. The nutrients, in liquid form, can be directly inserted into the apparatus and properly mixed. Then, they can go into circulation inside the system.

In order to improve the efficiency of the system, liquids can be distributed by plastic pipes. Their main characteristics are flexibility and transparency. The flexibility allows a simpler connection and reduces the load losses due to changes in direction, that would occur with the use of more rigid materials.

Moreover, a degassing apparatus is fundamental for microalgae cultivation. In fact, degassing is a process that removes a part of the oxygen produced by the organisms during photosynthesis from the culture liquid, necessary because this gas, in too high concentrations, has an inhibiting effect on growth. Because of the simplicity of our system, nutrient administration apparatus was used also as a degassing apparatus, by arranging suitable openings through which the liquid comes into direct contact with the external environment, uniformizing its pressure with the surrounding one.

An inoculation apparatus of gaseous substances was needed too. The main gaseous substance administered is the carbon dioxide, required for the metabolic processes of the photosynthetic organisms, assuring them the carbon necessary for the synthesis of the substances necessary for their survival and growth. Carbon dioxide is also fundamental to limit the phenomena of contamination by aerobic and anaerobic microorganisms, which cannot survive in environments with high percentages of CO₂.

The entire system of circulation of liquids and cultivation of microalgae was controlled through a system of monitoring of production parameters. This makes it possible to have all the data available to analyze the growth process of the organisms, and to evaluate the effectiveness of the changes, made during the cultivation phase, in order to program the future evolutions. The control allows corrective actions too, that can be carried out if there are changes in the parameters that go beyond the limits imposed. For example, the measurement of oxygen concentration allows to evaluate if the degassing systems are properly calibrated and if their functioning is correct, while measurements of pH allow to hypothesize the amount of nutrients present in the culture medium, assessing the rate of absorption by microalgae.

3.2.2 How it works

The culture liquid, consisting of water, microalgae and a solution of macro and micronutrients, is inserted into the nutrient administration apparatus, that is, in the case under examination, a simple tank. When the amount of liquid inside the tank reaches the set value (in this case, 80 L), the liquid handling apparatus is activated, and this device pumps the culture liquid inside the distribution apparatus. In this way, culture solution starts circulating inside the panel.

The liquid inside the panels follows the pre-established paths, created using the connection devices between the alveolar elements of the polycarbonate. These paths are chosen with the aim of maximizing light exposure time and reducing load losses, with the consequent

energy consumption connected to them. In addition to the path established within the various elements of the panel, another factor that maximizes light exposure is the high surface/volume ratio that flat panel photobioreactor allows to obtain.

At the end of this phase of exposure to light, the liquid is newly sent to the starting tank, where degassing phase occurs, and so a part of the oxygen produced during photosynthesis is released.

This regime corresponds to the cultivation and growth phase of microalgae, in which they use the elements dissolved in the liquid as nutrients, and synthesize them in substances necessary for their metabolism, thanks to the energy received through the light that irradiates them.

3.2.3 Technical drawings

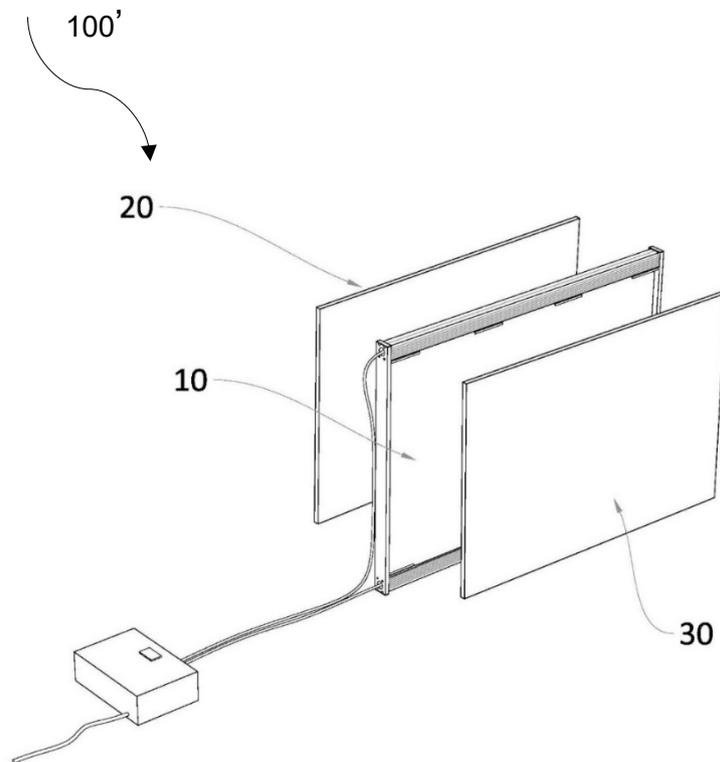


Figure 16: Schematic perspective representation of the embodiment of the apparatus, with two panels for the culture of microalgae and a bi-emissive optical illuminator

Where:

- 100' is the equipment for the cultivation of photosynthetic organisms
- 20 and 30 are the panels containing photosynthetic microorganisms

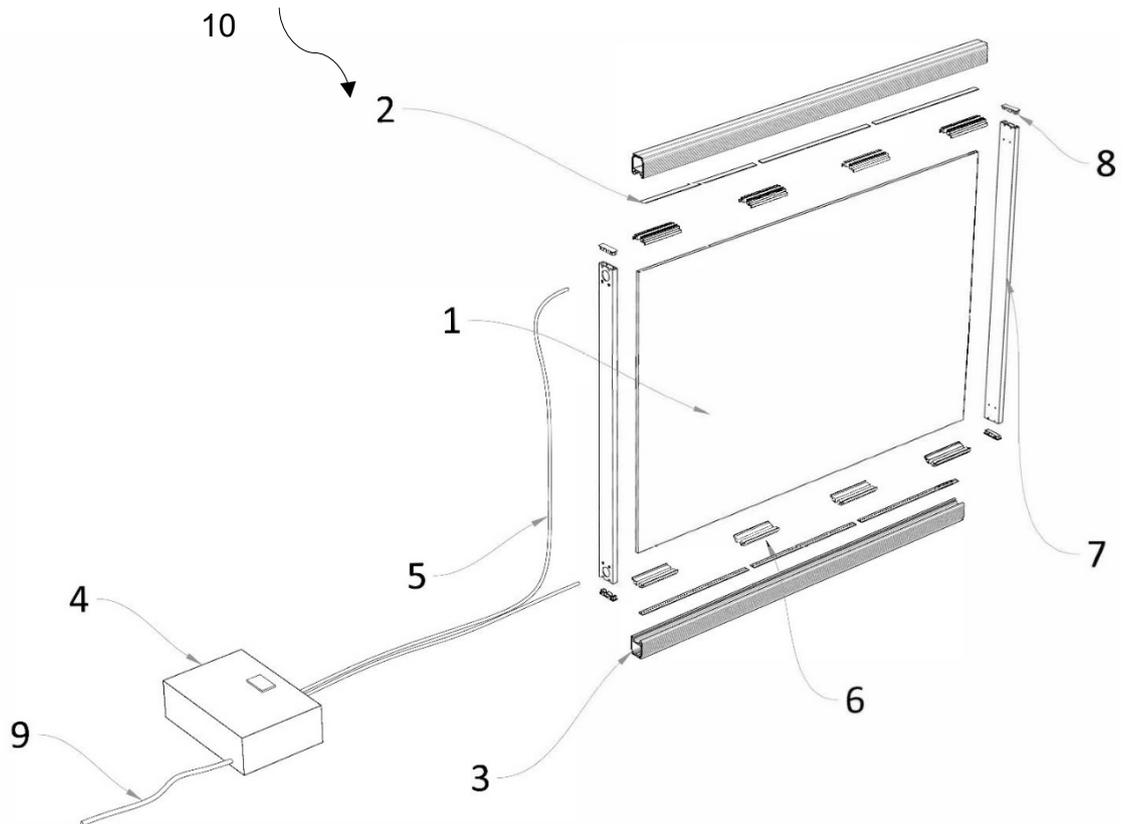


Figure 17: Schematic perspective representation of a bi-emissive optical illuminator according to the embodiment illustrated in Figure 16

Where:

1. optical guide
2. plurality of LEDs
3. a pair of profiles to dissipate the thermal energy generated by the LEDs
4. regulation and control framework
5. plurality of shielded cables
6. pair of supports for centering the optical guide
7. a pair of uprights to ensure the rigidity of the device for artificial lighting
8. closing caps to close the uprights
9. power supply system
10. bi-emissive optical device for artificial lighting.

Microalgae introduced into the panel come from precedent tuning tests. So, at the moment of the inoculation, they had an optical density of about 1.178. As a start point, the expected OD

should be about 0.25 - 0.3, so, a simple proportion is performed to determine which microalgal volume must be inoculated to reach this OD value as follow:

$$50 L : 1.178 = X : 0.3 \rightarrow X = 12.7 L$$

Where:

- 50 L is the volume of microalgae coming from the precedent tuning tests;
- 1.178 is the OD value previous to the inoculum;
- 0.3 is the expected OD value;
- X is the microalgal volume necessary to reach the expected OD.



Figure 18: Microalgae inoculation into the PBR

The panel configuration of this experiment has a capacity of 80 L, constituted thus by 12.7 L of microalgal volume, 640 mL of nutrients (400 mL of solution 1, 80 mL of solutions 2, 3, 4) and water, until the volumetric capacity of the system is reached.



Figure 19: Completed inoculation, off LEDs

The illumination system is constituted by six LED arrays, of which three in the superior part, and three in the inferior part of the photobioreactor. On every array, several types of LEDs are collocated. All these LEDs are governed by a controller, through different channels. During this experiment, only channel 5 and 10 had a non-zero value, because just white LEDs were used. The controller can supply LEDs with a minimum power of 0 to a maximum of 255; during the first part of our experiment, a power of 147 was provided to the LEDs. When microalgae growth assumed a constant trend, and no more growing, illumination conditions were changed, and supplied power became 255. Moreover, the lighting supplied had, in the first period, an intensity of 33 PAR, while this value increased to 57 in the second period. As already mentioned, PAR is the photosynthetically active radiation, which coincides, in this specific case (thanks to the spectroradiometer used, Oxycian PLA20, a device designed to measure the spectral power distribution of a source), with the micromoles of photons per square meter per second. Therefore, this quantity describes the flow of photons supplied to the microalgae.

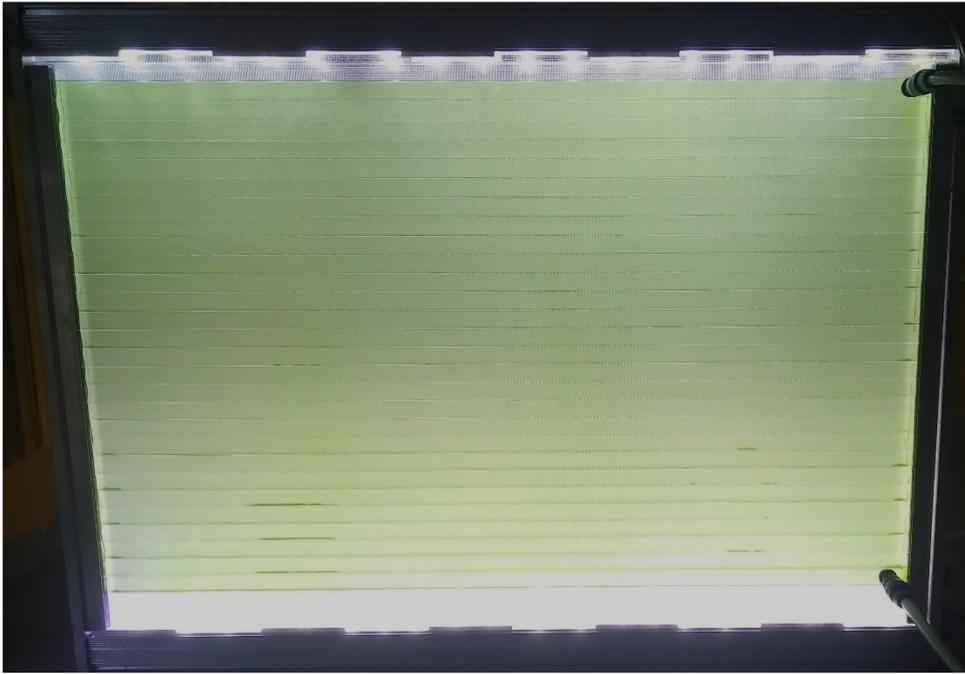


Figure 20: Three days after inoculation, LEDs are on

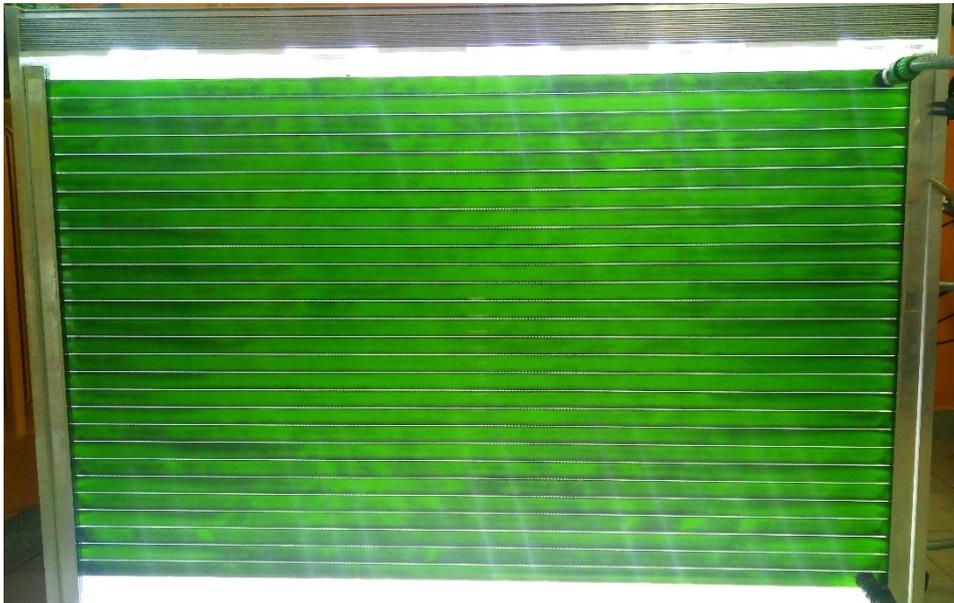


Figure 21: PBR after 36 days from the beginning of the experiment

These two precedent pictures clearly show microalgae growth: their colour, after 36 days from the start of the experiment, became darker compared to the beginning, and also fouling appeared, creating darker areas attached to the internal walls of the PBR. Fouling is mainly due to microalgae deposition or death.

The photobioreactor consumes 64 W when illumination system has an intensity of 33 PAR and only white LEDs are lit up, while it consumes 106 W when intensity has a value of 57 PAR. Instead, the electronics consumes about 44 W. The LED technology used guarantees a life expectancy of about 50,000 hours.

3.3 Data Collection

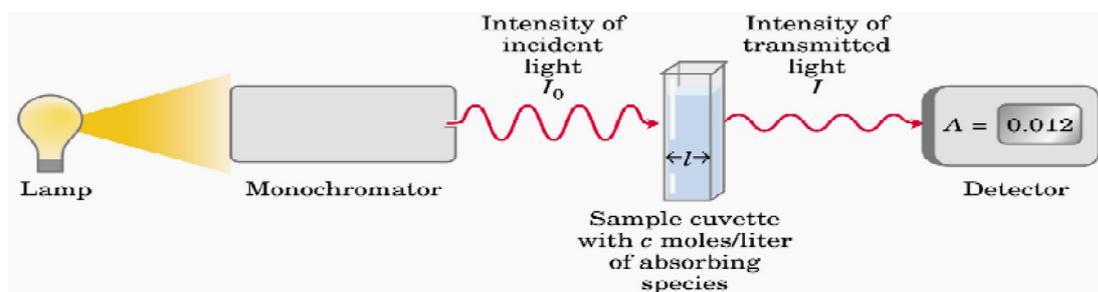
This paragraph is aimed at describing the main procedures developed during the experiment, in order to obtain the results reported in *Chapter 4*.

3.3.1 Growth Rate

Growth rate is measured in two different ways, linked to each other. The first measure is the *absorbance* (or *optical density OD*), a quantity used to describe the transmission of light through a specific liquid. In fact, optical characteristics of microalgae are fundamental parameters for analysing light field distribution in photobioreactors (PBRs). Moreover, when microalgae cells grow, their optical properties vary with growth time, because of the accumulation of pigment and lipid, cell division and metabolism. The optical density is described by Beer-Lambert law, which defines it as a logarithmic ratio between the light intensity incident on the liquid (I_{IN}) and the light transmitted through the liquid (I_{OUT}):

$$OD = -\log_{10} \frac{I_{OUT}}{I_{IN}}$$

Optical density was measured by a UNICAM Helios- γ spectrophotometer, that is an instrument capable of performing quali-quantitative analysis using a light source. The spectrophotometer uses the characteristic of light to be absorbed, at different frequencies, by chemical or biological substances.



Before measuring the absorbance of the sample, it is necessary to perform a blank test under the same conditions, omitting only the algal sample. Then, this last is introduced inside a cuvette, a small tube-like container (volume of 4 ml) with straight sides and a square cross section, made of a clear, transparent material such as plastic. Cuvette is then introduced inside the spectrophotometer, where a beam of light passes through the sample within the cuvette, to measure the absorbance of algal sample. Samples have normally several dilutions: pure sample, 50%, 25% and 12.5% (diluted with distilled water), and all these samples are duplicated. Dilution factor depends on the optical density of algal sample: when OD was low, pure sample, 50% and 25% dilutions were used. When OD became higher, with values of about 1.5, each sample was much more diluted, because microalgae shadow each other, and this badly affects the measure. Moreover, if absorbance values are higher than unit value, the line of the measure is not linear, and, so, measure is not reliable anymore. Measures are made at two different wavelengths:

- 680 nm (red), which is one of the two peaks of absorbance of *chlorophyll-a* (the other one is 430 nm, blue)
- 800 nm, in order to give an information about algal turbidity.



Figure 22: Spectrophotometer and cuvettes

The second quantity used for measure microalgal growth is *dry weight*, a measurement of the mass of microalgae when completely dried. Different strategies were used to find this quantity, often used together for make a comparison.

a – Crucibles and fan-assisted oven

This method is quite simple: three crucibles are filled up with a 10 ml volume of algal broth, they are weighted, using an analytical balance, (whose degree of precision of the decimal figures is 5) and then introduced into a fan-assisted oven, which reaches 105°C (378 K). These crucibles remain into the oven for at least 24 h. At the end of this period, crucibles are introduced inside a desiccator, a sealable enclosure containing desiccants, used for protect algae within crucibles from humidity. Here, crucibles cool down and, finally, they are removed and newly weighted, with the same balance.



Figure 23: a) On the left, fan-assisted oven, b) On the right, analytical balance



Figure 24: Desiccator with crucibles

b – Standard method

Microalgal volume (usually 10 ml) is poured on a paper filter (mesh 0.45 μm), which retains the microalgae and lets the excess water pass. Filters are rinsed with an excess of distilled water, before being dried; in this way, filter only contains microalgae, while salts are “washed away”. This process is done with the help of a vacuum pump, that aspires more water, and a Buchner funnel. Then, exactly like crucibles, filters are introduced in to the oven. After 4 hours they are removed and weighted, with analytical balance. This method is particularly indicated when algal broth contains a big amount of salts (contained in nutrients), that can affect dry weight if measured with classic method of crucibles.



Figure 25: Paper filter and Buchner funnel

c – Thermal balance

Microalgal volume (usually 10 ml) is poured on a paper filter inside a support, which is introduced inside a thermal balance. This instrument is used for the determination of the moisture content of sample, and it also provides the weight of sample after drying. At the beginning of the measurement, the moisture analyser determines the weight of the sample, which is heated until about 105°C (378 K) and the moisture vaporizes. During the drying process, the instrument continuously determines the weight of the sample and displays the reduction in moisture. When drying is finished, it is possible to see results in terms of moisture content and/or dry weight of algal sample.



Figure 26: Thermal balance

Although these methods are all generally valid, in the case under examination the most valid method was the one with the crucibles, which was used for dry mass determinations.

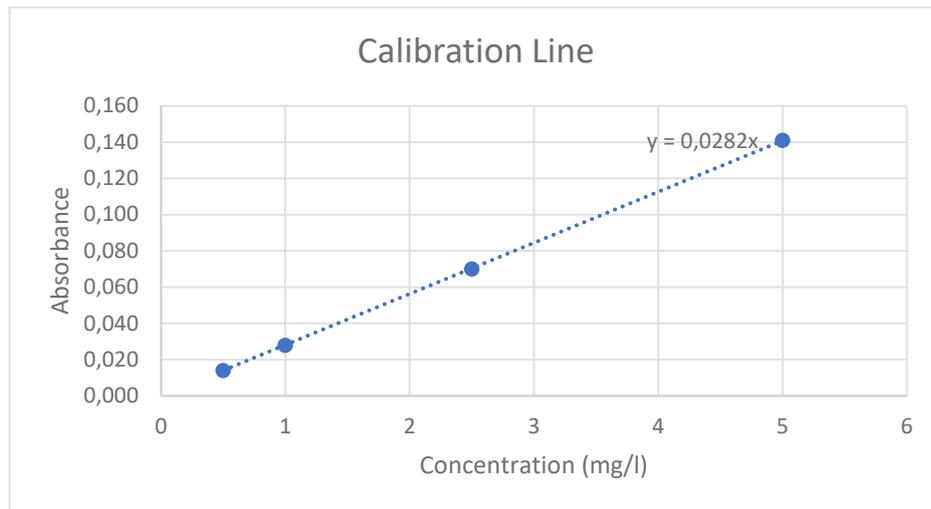
3.3.2 Nitrate measurement

Nitrates measurements were made with NANOCOLOR® Nitrati Z colorimetric test, whose measuring range is between 0.5 - 5.0 mg/L NO_3^- .



Figure 27: NANOCOLOR® Nitrati Z colorimetric test

A standard certified liquid, with nitrates concentration of 1000 g/l, was used. It was diluted 1:10, to obtain a stock solution with a concentration of 100 mg/l; from this stock solution, four standard volumes were obtained, and used for draw calibration line, through *Beer-Lambert law*, which relates light attenuation to the characteristics of the solution through which the light is passing.



Using angular coefficient of this line, microalgal samples can be analysed. Samples were taken from the tank, connected with PBR system, centrifugated with 10.000 rpm velocity and finally filtrated with a siring filter with pore size of 0.45 μm . Siring filter was used to minimize interferences, due principally to organic colloids, humic acids, oxidizing and reducing substances. Samples were further diluted with a factor of 400, to achieve significant results.

4. RESULTS AND DISCUSSION

4.1 Parameters trends

The most relevant parameters regulating microalgal growth are nutrients quality and quantity, pH, CO₂, turbulence and temperature. Turbulence is provided by a pump, which allows a continuous mixing. All the other parameters, and oxygen too, have been continuously monitored by a measurement line, through several probes, placed inside the tank. So, during all the period of the experiment, it was possible to collect all these measures. Then, these data were reported on an Excel sheet, creating different graphs, each one showing the trend of the mentioned parameters, as a function of time.

Carbon dioxide trend

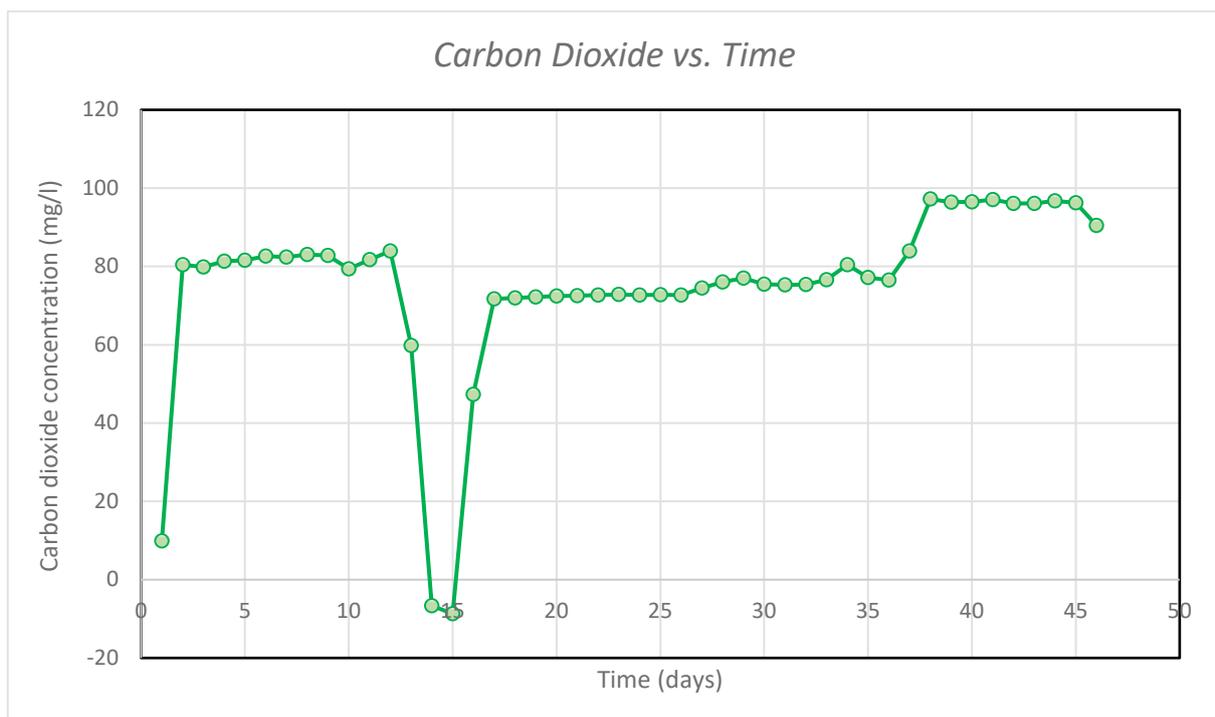


Figure 28: CO₂ as a function of time

The graph shows the average trend of CO₂ dissolved in water; so, it describes the quantity of available carbon dioxide within the system. It does not give information about carbon dioxide consumption, because separate tests are needed for evaluate this measure. On horizontal axis time is reported, measured in days, while on the vertical one CO₂ concentration is described, in mg/l.

Carbon dioxide is furnished by a tank, with a constant flow, comprised between 1.5 and 2.5 l/min. It is reasonable to distinguish three main phases, in which carbon dioxide trend is almost constant. In the first period, CO₂ values are around 80 mg/l, in the second 73 mg/l, while in the third, and last, period it is higher than the previous ones, around 97 mg/l. This is probably due to probe position: while in the first two periods it was inside the tank of the system, in the last period it was on the higher part of the tank, so the measure of available carbon dioxide was higher. As the graph shows, the only negative peak occurred on days 14 and 15, but this is simply due to the end of CO₂ tank, occurred during weekend. When the new carbon dioxide tank was introduced, CO₂ concentration newly started its growth.

Nitrates trend

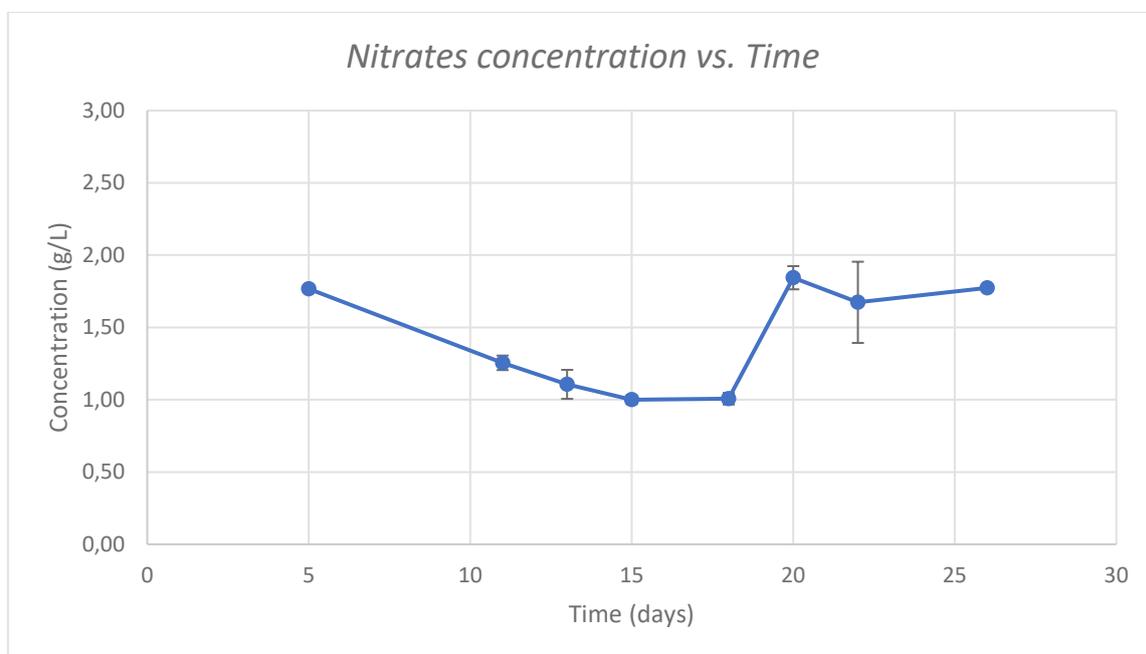


Figure 29: Nitrate concentration as a function of time

The graph shows the nitrates (NO₃⁻) concentration trend, as a function of time, within photobioreactor system. On the horizontal axis there is the time, measured always in days, while nitrates concentration is placed on the vertical axis, measured in g/l. These measures were made in a shorter period than the one of the experiment: in the first period, no instruments were available for this measure, while in the last period, some errors occurred with the kit used. This is clearly showed in the graph: while in the first period nitrates trend decreased, because of nutrients consumption by microalgae, in the second period this decrease did not happen, while

errors on measures increased. On day 18, a minimum nitrates concentration was reached, so, a new bottle of nutrients was added. This obviously provoked a rapid increase in nitrates concentration: the result is a positive peak, on day 20, in which nitrates concentration reached its higher value, equal to 1.84 mg/l.

Oxygen trend

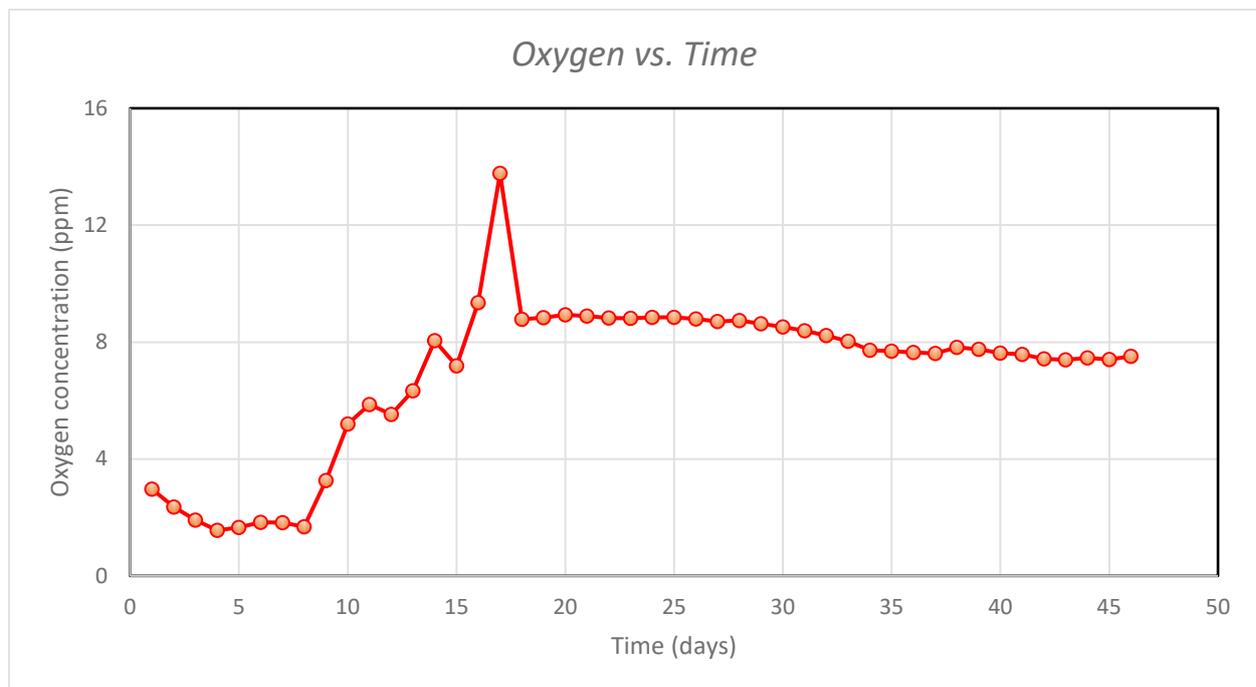


Figure 30: Oxygen concentration as a function of time

The graph shows the oxygen production curve, that describes the quantity of oxygen within photobioreactor system, produced by microalgae during their photosynthesis. On the axis there are time (on the horizontal one), measured in days, and oxygen concentration (ppm, on the vertical one). While carbon dioxide is consumed by microalgae during photosynthesis, oxygen is produced. Because of the oxygen production, this kind of photosynthesis is also called “oxygenic”: in this process water is the electron donor and, after hydrolysis, it releases oxygen. This can be described through a general equation:



Oxygen monitoring started on day 17. As the graph shows, in fact, the values before of this data are affected by many errors, and they are very different from the ones of the second period. From day 17, a probe with optical revelation was installed, and from this point oxygen concentration values became constant, with a value around 8.70 ppm, even if they undergo a

slow decrease, which becomes more evident from the 37th day, when illumination conditions changed, and light intensity increased.

Temperature and pH trends

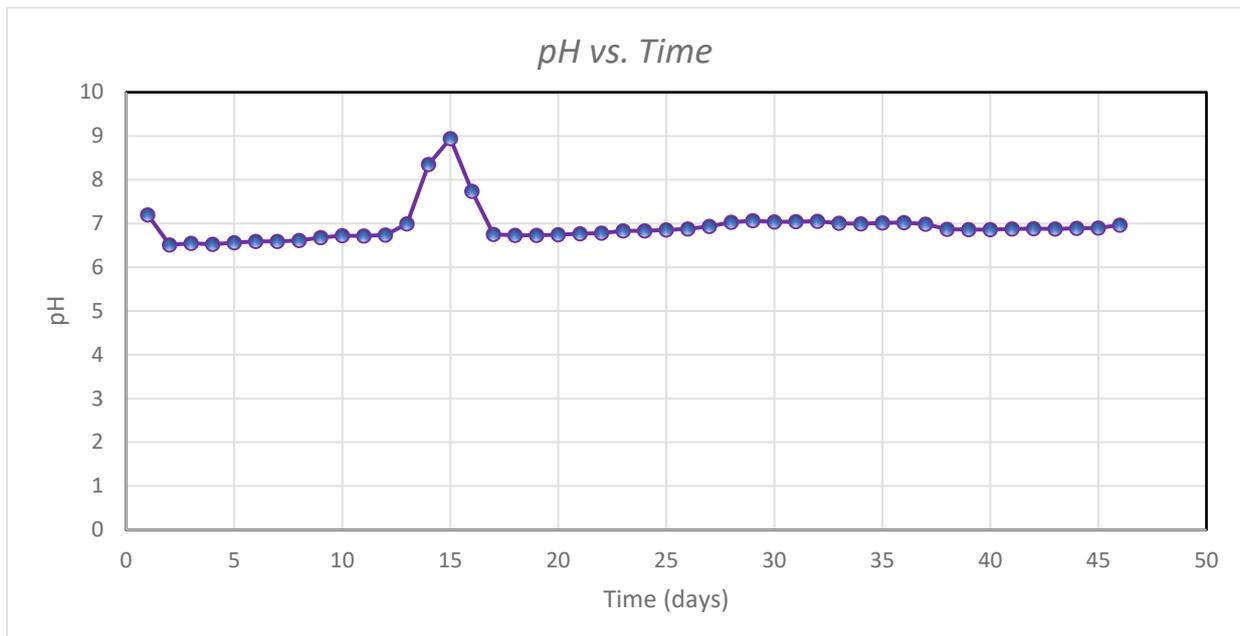


Figure 31: pH as a function of time

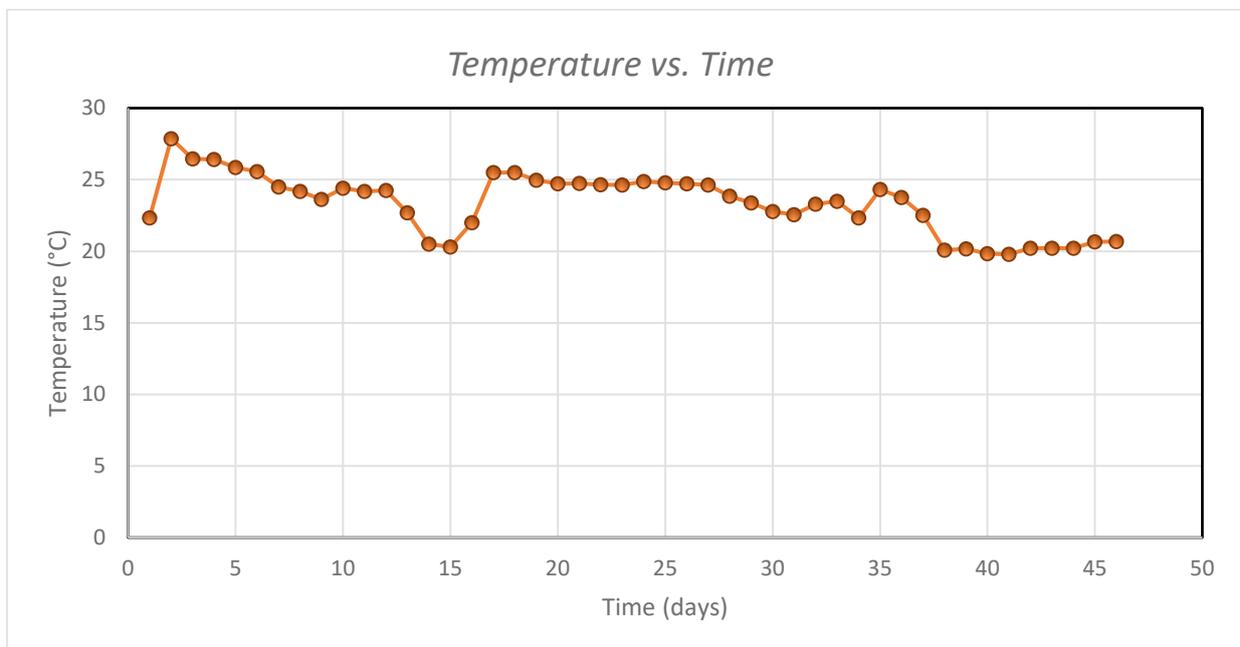


Figure 32: Temperature as a function of time

The graphs show temperature and pH trends as function of time. So, time is reported on the horizontal axis, while temperature (measured in °C) and pH are on the vertical ones. The

pH range for most cultured algal species is between 7 and 9, with the optimum range being 8,2 - 8,7. In the case under examination, pH does not undergo particular variations: it remains roughly constant, comprised in a range between 6.5 and 7. Moreover, pH is influenced by CO₂: on day 15 a positive peak occurs, because of the total absence of CO₂. When CO₂ is newly introduced, pH returns to the previous values.

On the other hand, temperature undergoes some variations, but it remains substantially included in an interval around the temperature of 25°C. So, temperature was not limiting in microalgal growth.

4.2 Growth trends

4.2.1 *Optical densities vs. Time*

The growth of an axenic culture of microalgae is generally characterized by five phases. The first one is the lag or induction phase, in which cell density has a little increase. This phase is due to the physiological adaptation of the cell metabolism to growth. To follow, there is the exponential phase: here, the cell density increases as a function of time t according to a logarithmic function:

$$C_t = C_0 \cdot e^{mt}$$

where C_t and C_0 are the cell concentrations at time t and 0, respectively, and m = specific growth rate. The latter is mostly dependent on algal species, light intensity and temperature. In the third phase declining growth rate occurs: cell density starts to slow down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth. The following one is the stationary phase, in which the limiting factor and the growth rate are balanced: cell density becomes roughly constant. The fifth and last phase is the “crash phase”, or death: cell density rapidly decreases, while the culture eventually collapses.

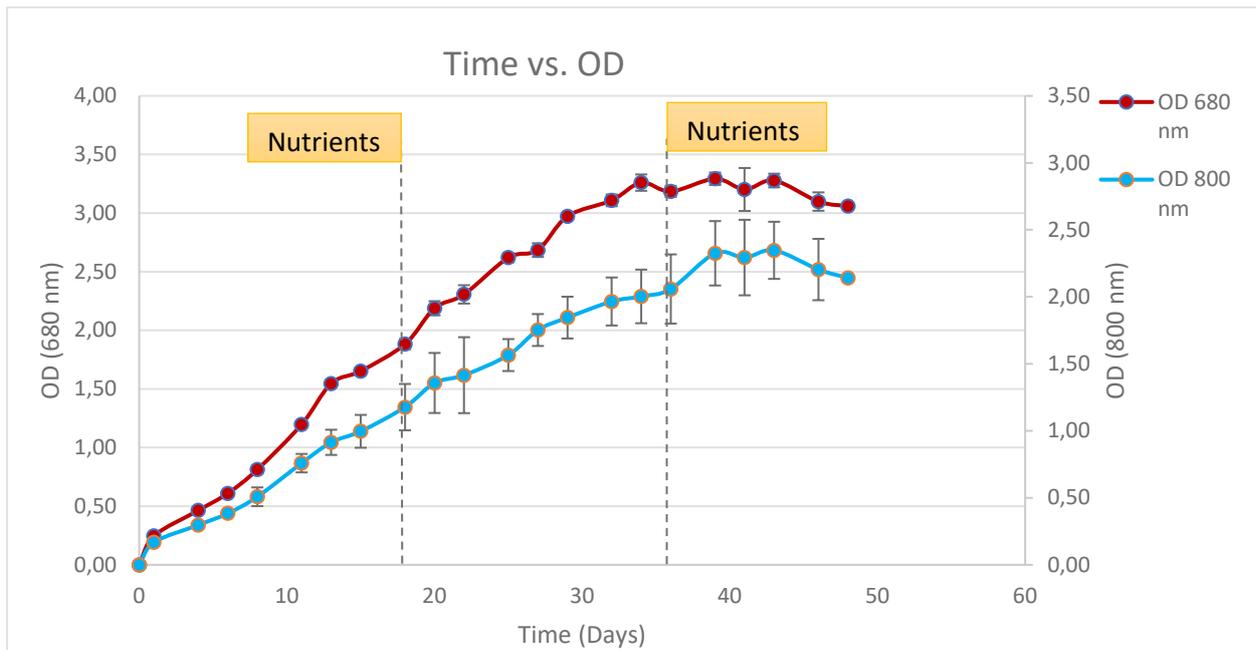


Figure 33: Microalgae growth curve

This graph shows the growth rate curve of *Scenedesmus Obliquus*. On horizontal axis time is reported, measured in days, while on the vertical one the optical density (OD) is reported, both at 680 and at 800 nm.

Microalgae growth is mainly described by the curve at 680 nm. On the first day of the experiment, microalgae, nutrients and water were introduced into the tank, linked to the PBR. Power supplied to the LEDs was 147, while a 33 PAR intensity value was established. In this experiment, lag phase did not occur. In fact, as the graph shows, optical density immediately started its growth in exponential way. But the other phases too are not well represented: microalgal growth has continued for much longer than expected, so, the third and the fourth phases do not appear on the graph. In fact, the values have never stopped increasing, at most, they remained constant, but this is probably due to the lack of algae mixing and their deposit on the walls (fouling). Nutrients, which should have been added when the curve had become constant, were actually added on the 18th day of the experiment, when the trend was still growing. The addition of nutrients did not result in any substantial change in the trend, which remained approximately unchanged. At the 36th day of the experiment, the curve suffered a decrease for the first time, therefore, another bottle of nutrients was added: this addition did not provoke a significant increase; for this reason, illumination conditions were changed, setting the power and intensity values respectively to 255 and 57 PAR. Increasing the light did not produce the expected effects: trend did not increase in a significant way, while a lag phase occurred.

The curve at 800 nm describes turbidity into microalgal samples. As the graph shows, the two curves are almost parallel; therefore, as the microalgal growth increases, the turbidity also increases, in an almost proportional way. Moreover, this curve is mostly linear, similar to a straight line.

Exponential phase lasted about 13 days. After this period, optical density at 680 nm continued its growth, but more linearly. The higher value reached by this quantity, during the exponential period, was 1.547, while the higher value reached during the entire experiment period, was 3.293, on day 39. So, at the end of the experiment, a value greater than twice the maximum obtained at the end of the exponential growth period was reached. Therefore, this underlines that microalgae growth has not respected the five stages of growth, but, on the other hand, they have grown more than expected, reaching higher values than those of previous experiments.

A second important result is related to the light intensity used. The experiment foresaw reaching high microalgal densities increasing the light intensity from 33 to 57 PAR. Instead, increasing light did not provoke significant effect, but, on the contrary, it has simply confirmed the previous lag phase, in which optical densities remained roughly constant, around a value of 3,2 (at 680 nm). But this result is still positive, because the expected values have in any case been reached, but with a lower light intensity. As a consequence, consumption and therefore energy costs were lower: it is reasonable to state that the expected results have been achieved in conditions of energy efficiency.

4.2.2 *Dry weight vs. Time*

Dry weights were calculated through crucibles and oven method. With this method, the measurements obtained have been largely influenced by the presence of salts. In fact, BG-11 medium contains a big amount of salts, and every addition of nutrients provoked a sharp increase of dry weights. So, these measurements are overestimated, and especially the highest values obtained are not truly indicative of the real dry weights, but they are nevertheless a useful index to understand the microalgal growth.

Moreover, these quantities are useful for the calculation of *specific growth rate*, which is a measure of how fast microalgae grow, through the following formula:

$$\mu = \frac{\ln(x(t_1)) / \ln(x(t_0))}{t_1 - t_0}$$

Where $x(t_1)$ and $x(t_0)$ are dry weights calculated on days t_1 and t_0 . The most suitable periods on which to calculate the growth rate are those far from the addition of nutrients, and with a dry weight trend proportional to the OD one. For these reasons, two periods were chosen: the first from day 11 to day 15, the second one from day 29 to day 34. Another key parameter, necessary to understand microalgal growth, is the *overall biomass productivity*, which can be calculated via the following equation:

$$P_{overall} = \frac{\Delta x}{\Delta t}$$

Where Δx is the variation of biomass concentration (mg L^{-1}) within a cultivation time of Δt (D).

Growth rates and overall biomass productivities were calculated in both the periods, and reported in *Table 4*:

Period (days)	Growth Rate (D^{-1})	Overall Biomass Productivity ($g L^{-1} D^{-1}$)
From day 11 to day 15	0,094043	0,099553
From day 29 to day 34	0,067538	0,366858

Table 4: Growth rate and overall biomass productivities

As shown in the table, the highest value of specific growth rate was obtained in the first period; in particular, this value was reached at the peak of the exponential growth, described through OD curve. On the other hand, the highest overall biomass productivity was reached during the second period. During these two periods, about 7.96 and 29.35 g of microalgae were produced, respectively, in one day, within the photobioreactor system.

As mentioned above, increasing light did not produce any significant effect, neither regarding to dry weight.

4.2.3 Correlation OD – light intensity

In this experiment, only two light intensities were used: the first one of 33 PAR, and the second one, 57 PAR. However, before setting the light to 33 PAR, microalgae were subjected to a light intensity of about 12 PAR, in order not to create in them an excessive stress, linked to the sudden intense lighting. As already mentioned, 57 PAR light intensity did not provoke any

effect on microalgal growth, but, instead, it just provoked a lag phase. So, an intensity value of 33 PAR, applied for 34 days, was considered a limiting factor for microalgal photosynthetic growth. Therefore, considering only the period in which the luminous intensity was 33 PAR, it is possible to obtain an increasing curve, whose trend is approximately linear, and it is possible to find its equation.

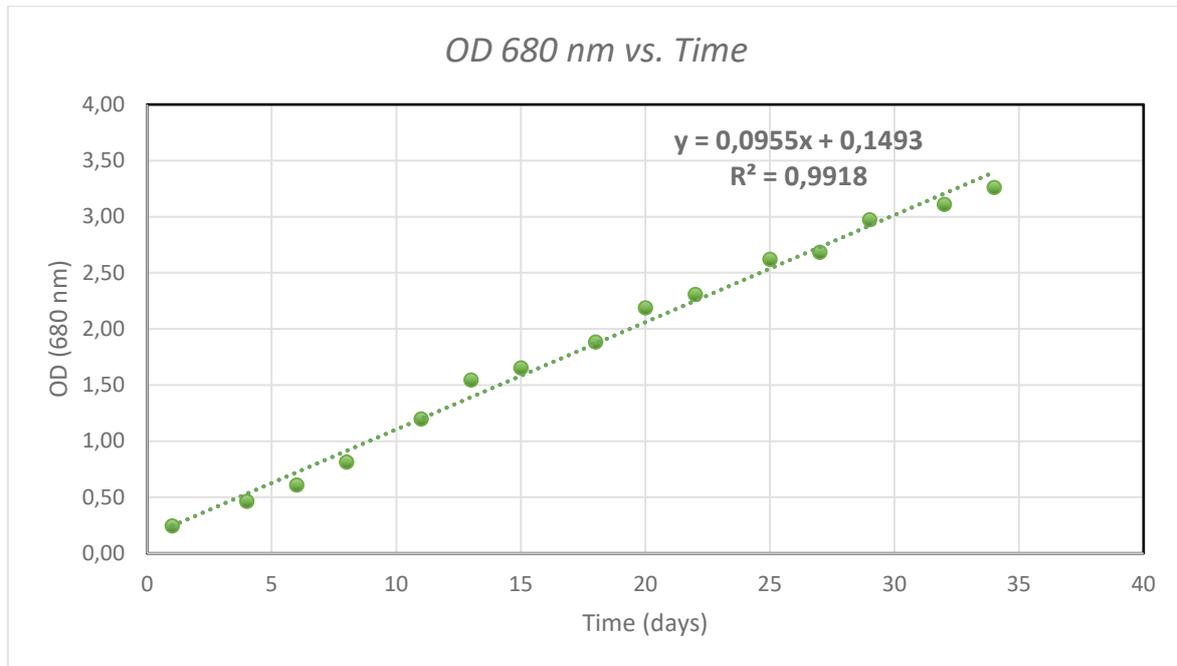


Figure 34: Optical densities at 680 nm as a function of time

On the other hand, it would be interesting to identify a relationship between the light intensities (PAR) and the OD values obtained. Considering the maximum value reached with the use of 33 PAR, equal to 3.26, it is possible to establish a proportion:

$$33 \text{ PAR} : 3.26 = 1 \text{ PAR} : F$$

$$F = 0.100$$

Where F is the value of the optical density resulting from the application of a luminous intensity of 1 PAR.

This result could be useful for identifying what is the optical density resulting from a given value of light intensity applied, expressed in PAR, simply multiplying this intensity by the factor 0.100.

On the other hand, if the objective is to reach a certain optical density value, then, it is sufficient to multiply this value by the reciprocal of F. This last method was applied to the

optical densities resulting from this experiment: each OD value, obtained at 33 PAR, was multiplied by $1/F$, to identify the light intensities required to obtain it. The results are reported on the graph below:

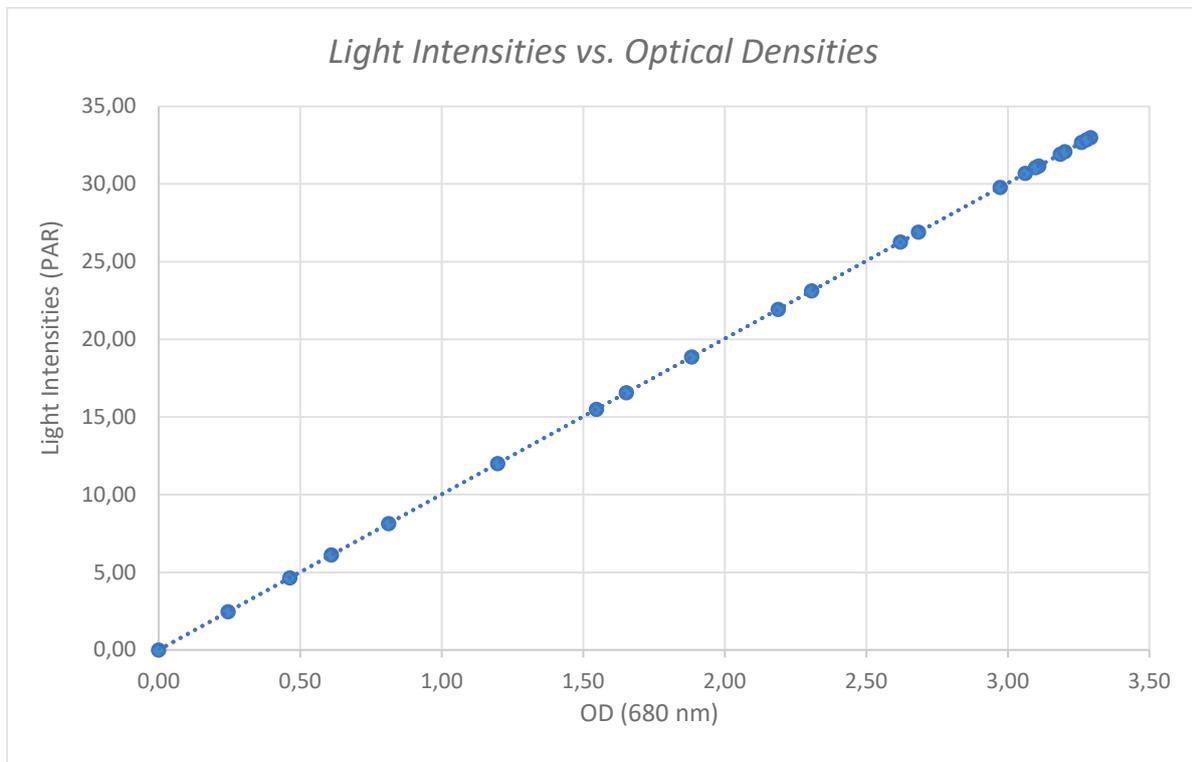


Figure 35: Correlation OD and light intensities

In this way, to reach the OD values obtained by this test, it may be possible to use less than 33 PAR.

Through this method, a linear correlation between optical densities, found during this experiment, and light intensities, measured in PAR, was found. It would be interesting to make a step test, in which light intensity does not assume a constant value, but increases by successive steps, until 33 PAR value is reached. In this way, the experiment would last the same period, but, because light intensities would be lower than the one used, also energy consumption would be lower. Such a similar experiment would take to comparable results, but with lower costs, than the ones of this thesis work.

5. Conclusions

The principal purposes of this thesis work were mainly two. The first one was to indagate microalgal growth under specific conditions, using LED illumination and monitoring all the process parameters; the second purpose was to individuate coefficients that will be used in the future to proportionally calibrate the light intensity related with biomass density of PBR.

Regarding the first aim, this thesis work produced different results than expected. In fact, the starting idea was to increase the light intensity from 33 to 57 PAR, observing the consequent growth, and obtaining high OD values. Indeed, the increase in light intensity did not produce significant increase in OD, but, on the contrary, it led to a lag phase and a substantial stop in microalgae growth. On the other hand, the expected OD values were in any case achieved, but using a lower light intensity than the expected one, equal to 33 PAR. This is an important goal in terms of energy efficiency. By observing just the two periods chosen, between days 11-15 and 29-34, it was possible to obtain indicative values of the specific growth rate and the overall biomass productivity, which reached higher values than those expected.

It is possible to make a comparison between the results obtained and the ones found in literature, in several articles. Considering the period from day 29 to day 34, overall biomass productivity reached a very high value. This value, equal to $0.367 \text{ gL}^{-1}\text{d}^{-1}$, is higher than those found, for example, by Thiansathit and Ho in their researches, "The kinetics of *Scenedesmus obliquus* microalgae growth utilizing carbon dioxide gas from biogas" (2015) and "Scenedesmus obliquus CNW-N as a potential candidate for CO₂ mitigation and biodiesel production" (2010) respectively. In fact, in the first article, a biomass productivity equal to $0.145 \text{ gL}^{-1}\text{d}^{-1}$ was achieved, using fluorescent lamps, whose intensities were $80 \mu\text{mol s}^{-1}\text{m}^{-2}$. Instead, in the second reported research, a value of $0.293 \text{ gL}^{-1}\text{d}^{-1}$ was obtained, using tungsten filament lamps, having an intensity of $60 \mu\text{mol s}^{-1}\text{m}^{-2}$. In the case under examination, a higher productivity was achieved, using, for more, LEDs, whose consumption is lower than other light sources, with lower intensity. In fact, using only white LEDs, the photobioreactor consumes 64 W when light intensity is equal to 33 PAR, while it consumes 106 W when intensity is 57 PAR. Instead, the electronics consumes about 44 W. A comparison has been done, using both LEDs illumination and neon lamps, and light intensities equal to 33 and 57 PAR. Regarding neon lamps, two configurations were used: the first, with 3 neon lamps (58 W each), and a light intensity of 33 PAR, the second with 5 neon lamps (58 W each), and a light intensity of 57 PAR. Results are reported below:

Light Source	3 Neon	5 Neon	LED	LED
PAR	33	57	33	57
Electric consumption (W)	174	290	64	106
Biomass growth in 5 days (%)	43		66	

Table 5: Comparison between different light sources

Table 5 shows that, under the same conditions of light intensities, LED system consumes very less than traditional illumination system. Moreover, this reduction does not provoke a reduction in biomass production, because LED system, in 5 days of observation, produces more biomass than the traditional system. This comparison has been done using only white light, because traditional neon systems do not allow to influence spectrum composition.

Regarding the second purpose, it was possible to find the optical density produced by the application of 1 PAR. Through this proportion, it would be possible, established an optical density value, to find the light intensity necessary to obtain it. On the other hand, it would be also possible to find the optical density resulting by the application of a given value of light intensity, measured in PAR. Because these results are theoretical, it would be interesting to perform step tests, in which different values of increasing light intensity would be used, to observe the effects of each successive step of light increase. If the results are satisfactory, it means that the results obtained by immediately setting the value of 33 PAR could be reachable also with variable, but minor, intensities. Obviously, this would comport a lower energy consumption, and, consequently, lower costs.

So, the use of LEDs has proved to be very effective, both because they have allowed to achieve very high growth values, and because this objective has been achieved with a rather low light intensity. This led to lower consumption than expected. Finally, the use of LEDs illuminator has led to better results than the use of simple neon lamps.

The most important results of this thesis work are the unexpected growth values under the illumination conditions imposed. This experiment could be the first step in a series of tests aimed at increasing microalgae under conditions of ever better energy efficiency. The maximum OD value achieved, equal to 3.26, and energy conditions make this system optimal for microalgal growth. The use of LEDs illumination makes this system suitable for a scale-up too. In fact, the only precedent realization of a similar system was realized in 2013, to carry out various activities, but only six months later was decided to return to the research and

development phase. The reason was mainly economic: photobioreactor was illuminated by neon lamps, whose energy consumption were too high.

To have more scientific correctness, this experiment will surely be repeated in the future, to verify the results obtained. Other experiments involving the use of neon lights are planned, to make a more accurate comparison with LED illumination. As already mentioned, it would be interesting to perform a step test, to assess if the theoretical results obtained with this work are also valid from the experimental point of view.

In the last decades, numerous steps have been taken in the world of microalgae. Now that the results of climate change are increasingly evident, it is essential to continue to deepen research in this field. Before microalgae can be used on a large scale, a long research phase is still required, more researches, more experiments must be conducted in order to identify the best conditions for their cultivation, both physically and biologically, and economically. When the best solution will be identified, microalgae could contribute significantly to the abatement of the carbon dioxide emitted, at least from the main stationary sources.

REFERENCES

- *Basu et al.* “Operational strategies for maximizing CO₂ utilization efficiency by the novel microalga *Scenedesmus obliquus* SA1 cultivated in lab scale photobioreactor”
- *Bhola et al.* “Overview of the potential of microalgae for CO₂ sequestration”
- *Blanken et al.* “Cultivation of microalgae on artificial light comes at a cost”
- *Blanken et al.* “Predicting microalgae growth”
- *Cheirsilp et al.* “Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation”
- *Chen et al.* “Application of experimental design methodology for optimization of biofuel production from microalgae”
- *Chini Zittelli et al.* “A Modular Flat Panel Photobioreactor (MFPP) for indoor mass cultivation of *Nannochloropsis* sp. under artificial illumination”
- *Chisti*, “Biodiesel from microalgae beats bioethanol”
- *Chu*, “Biotechnological applications of microalgae”
- *Franchino et al.* “Growth of three microalgae strains and nutrient removal from an agro zootechnical digestate”
- *Frac et al.* “Microalgae for biofuels production and environmental applications: A review”
- *Glemser et al.* “Application of light-emitting diodes (LEDs) in cultivation of phototrophic microalgae: current state and perspectives”
- *Ho et al.* “Effect of light intensity and nitrogen starvation on CO₂ fixation lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N”

- *Ho et al.* “Photobioreactor strategies for improving the CO₂ fixation efficiency of indigenous *Scenedesmus obliquus* CNW-N: Statistical optimization of CO₂ feeding, illumination, and operation mode”
- *Krujatz et al.* “MicroLED-photobioreactor: Design and characterization of a milliliter-scale Flat-Panel-Airlift-photobioreactor with optical process monitoring”
- *Indira Priyadarshani and Biswajit Rath*, “Commercial and industrial applications of micro algae – A review”
- *Lehmuskero et al.* “Light and photosynthetic microalgae: A review of cellular- and molecular-scale optical processes”
- *Liao et al.* “Physiological-phased kinetic characteristics of microalgae *Chlorella vulgaris* growth and lipid synthesis considering synergistic effects of light, carbon and nutrients”
- *Mahmoud et al.* “Evaluation of the potential for some isolated microalgae to produce biodiesel”
- *Meier et al.* “Photosynthetic biogas upgrading using microalgae: Effect of light/dark photoperiod”
- *Miranda et al.* “Physical-energy characterization of microalgae *Scenedesmus* and experimental pellets”
- *Murakami et al.* “The biological CO₂ fixation and utilization project by rite”
- *Nedbal et al.* “Experimental validation of a nonequilibrium model of CO₂ fluxes between gas, liquid medium, and algae in a flat-panel photobioreactor”
- *Pavlik et al.* “Microalgae cultivation for carbon dioxide sequestration and protein production using a high-efficiency photobioreactor system”
- *Qiu et al.* “Effects of pH on cell growth, lipid production and CO₂ addition of microalgae *Chlorella sorokiniana*”
- *Razzak et al.* “Integrated CO₂ capture, wastewater treatment and biofuel production by microalgae culturing—A review”
- *Reyna-Velarde et al.* “Hydrodynamic and mass transfer characterization of a flat-panel airlift photobioreactor with high light path”

- *Sierra et al.* “Design scenarios for flat panel photobioreactors”
- *Singh et al.* “Development of suitable photobioreactor for algae production – A review”
- *Shultze et al.* “Light emitting diodes (LEDs) applied to microalgal production”
- *Slegers et al.* “Design scenarios for flat panel photobioreactors”
- *Tamburic et al.* “Design of a novel flat-plate photobioreactor system for green algal hydrogen production”
- *Ugwu et al.* “Photobioreactors for mass cultivation of algae”
- *Verma et al.* “Carbon dioxide sequestration and its enhanced utilization by photoautotroph microalgae”
- *Wishkerman et al.* “Application note: A novel low-cost open-source LED system for microalgae cultivation”
- *Yan et al.* “Photosynthetic CO₂ uptake by microalgae for biogas upgrading and simultaneously biogas slurry decontamination by using of microalgae under various light wavelengths, light intensities, and photoperiods”
- *Yan et al.* “The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae *Chlorella* sp.”
- *Zhang et al.* “Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor”
- *Zhao et al.* “Temporal scaling of the growth dependent optical properties of microalgae”
- *Zhou et al.* “Bio-mitigation of carbon dioxide using microalgal systems: Advances and perspectives”
- *Zhuang et al.* “The characteristics and influencing factors of the attached microalgae cultivation: A review”