**Scale-Up of a Fibonacci-type photobioreactor for the production of *Dunaliella salina***

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# Abstract

In this work, the previously proposed Fibonacci-type photobioreactor is scaled up and evaluated to produce *Dunaliella salina.* First, the composition of the culture medium was optimized to achieve maximal productivity. Next, the Fibonacci-type reactor was scaled up to 1,250 L maintaining high solar radiation interception capacity of this type of reactor. Finally, the performance of the reactor for the production of green cells of *Dunaliella salina* at the environmental conditions prevailing in the Atacama Desert was evaluated. Data demonstrated that the proposed photobioreactor allows the temperature, pH and dissolved oxygen concentration to be maintained within the optimal ranges recommended for the selected strain. Both better exposure to solar radiation and photonic flow dilution avoids the use of cooling systems to prevent overheating under outdoor conditions. The system allows up to 60% more solar radiation to be intercepted than does the horizontal surface. Likewise, allowing to maintain the pH efficiently through CO2 Injection and to keep the dissolved oxygen concentration in acceptable ranges, thanks to its adequate mass transfer capacity. The biomass concentration reached up to 0.96 g·L-1, three-times higher than that obtained in a raceway reactor under the same environmental conditions, whereas productivity was up to 0.12 g/L·day (2.41 g/m2·day). Maximum specific outdoor growth rates reached up to 0.17 day-1. Undoubtedly, this technology scaled up constitutes a new type of photobioreactor for use at the industrial scale since it is capable of maximizing biomass productivity under high light conditions.

Introduction

Microalgae are an important source of food, especially in Asian countries such as China, Japan and Korea. Standing out amongst these are the green algae (Chlorophyceae): *Chlorella vulgaris, Haematococcus pluvialis, Dunaliella salina,* and the cyanobacteria*, Spirulina maxima.* These microalgae are widely used as nutritional supplements for humans and as feed additives [1]. They are also extensively used in the pharmaceutical and nutraceutical fields; this has promoted the biotechnological development of microalgae through sophisticated cultivation and screening techniques in order to satisfy the rigorous demands of the market [2]. Amongst the nutraceutical components, pigments are the most commercially valued [3, 4]. *Dunaliella salina* is the best commercial source of natural β-carotene compared to other sources [5]. The global β-carotene market was valued at 510 million dollars in 2018 and will reach 520 million dollars by the end of 2025 [6].

*Dunaliella salina* is characterized by a high protein (57%) and carbohydrates (32%) content, but a low biomass productivity of 0.22-0.34 (g·L-1 day-1), it being produced in two phases (green and red) to produce large amounts of β-carotene [7, 8]. This microalga tolerates wide pHs, from 5.5 to 10, although pH 7 is optimal for growth (green phase) whereas pH 9 is optimal for producing β-carotene (red phase). It can grow in a wide temperature range, between 0 and 45 °C, and its mobility ceases at -18 °C; however, its optimum growth range in laboratory cultures is between 25-35 °C [5]. Growth of *Dunaliella* *salina* at different irradiances, from 50 to 3,000 μE·m-2·s-1 has been reported, optimal irradiance ranging within the 800-1,500 μE·m-2·s-1 range [9, 10].

*Dunaliella salina* is mainly produced in raceway reactors, it provides scarce control of culture parameters, then both quality and productivity of the biomass from these reactors being low. To increase it, the culture conditions must be better controlled. In this regard, closed tubular photobioreactors satisfy these conditions although the production costs are higher than in open systems [11–13]. It should be noted that the photobioreactor design, its geometry and its geographical location, are the main factors influencing microalgae biomass productivity. For this reason modelling and simulation have been utilized to improve photobioreactor design by considering the daily variations in the culture parameters that influence productivity [14–16]. Close tubular photobioreactors need a temperature control system to cool or heat them, which can be expensive. Furthermore, to improve light availability in the photobioreactors, the relationship between the illuminated surface area/volume and the light penetration length must be optimized [17]. When scaling up a reactor, conditions are easily controlled at the small scale but problems can arise leading to commercial failures in microalgae production at a large scale; for example, obtaining an adequate light availability and temperature are essential for the efficiency of microalgae but it is also a function of both sufficient CO2 supply and O2 oxygen removal [18–21].

To achieve maximum productivity maximum light availability must be provided, in addition to different parameters such as nutrient concentration, temperature, pH and dissolved oxygen must be controlled; however, not all photobioreactors can perform this efficiently. The Fibonacci-type photobioreactor solves many of these problems since it uses concepts emanating from nature where biological processes are exposed to daily light and darkness cycles [22]. As brightness intensity is variable, it is necessary to adjust the photoperiod and light intensities to avoid stress and photoinhibition in the microalgae [23, 24]. The Fibonacci-type photobioreactor (CL. Patent 48664) possesses favourable characteristics for the cultivation of microalgae such as: (i) the separation between each pipe coil (the pitch) allows adequate cooling of the liquid medium by advection and convection of the surrounding air in the temperate sectors [25]; (ii) the illuminated surface is controlled by its height and the angles of exposure to solar irradiation, thus allowing the dilution of photonic flow [22]; (iii) its vertical helical configuration (constant pitch, variable radius and upward flow) allows excellent gas exchange that ensures maximal CO2 fixation as well as adequate O2 removal [22].

*Dunaliella salina* is traditionally cultivated on raceway pond reactor, a system that has performance problems in Atacama Desert, as it is exposed to high rates of evaporation, where water is scarce. In addition, sandstorms and winds usually occur during the year that constantly contaminate crops, therefore the need to explore in tubular photobioreactors. The objective of this work is to scale-up the Fibonacci photobioreactor for the outdoor production of green cells of *Dunaliella salina* under the climatic conditions present in the "Pampa del Tamarugal" region of the Atacama Desert, northern Chile. Culture media based on natural nutrient sources were tested. The performance of the reactor was evaluated indoor, next it being scaled-up and evaluated outdoor for producing *Dunaliella salina*. The reactor surface angles were changed to improve the light interception. The illuminated surface to volume ratio was also improved. The successful demonstration of this new concept reactor confirms that the technology is sufficiently effective for scaling up to the industrial scale.

Materials and Methods

## Microorganisms and culture conditions

The microalgae strain *Dunaliella salina* (code 007) was used. This strain is available at the culture collection of the company GATTAVARA SACI, and it was isolated from the Salar Atacama Desert (Log. 68º 16’13.5’’ O: Lat. 22º53’51.6’’ S). The culture media utilized were based on the medium already proposed [8]. Four different culture media were evaluated under laboratory conditions in a 6 L Fibonacci photobioreactor all of them using KNO3 as nitrogen source. The culture media was proposed by the company (Table 1Table 1). The aim was to select the most adequate for large scale production, where the nitrogen source is KNO3 of agricultural origin, providing the highest growth rate. The reactor was inoculated at a cell density of 5·105 cells/ml - equivalent to 0.3 g·L-1, then it being operated in batch mode to evaluate the performance of the cultures when using the different culture media. The optimal culture media was utilized on experiments performed at a larger scale.

## Scaling up of the Fibonacci-type photobioreactor and operating conditions

The Fibonacci-type photobioreactor has been scaled up at a site in the Atacama Desert where temperatures fluctuate between -5 to 40 °C, with an average of 330 clear days per year. The average maximum solar radiation is 1,752 μE·m-2·s-1 [26]. The Fibonacci-type photobioreactor already reported, with a capacity of 330 L, was utilized [22]. On the basis of this design the exposure angle on the spiral-tube surface was adjusted to the latitude and longitude of the geographical location where it was to operate (Tarapacá, Chile) in order to achieve the maximum light exposure [27]. The reactor surface to volume ratio was 50 m-1 (S/V), whereas the tube surface exposed to light (St) to volume ratio was 77 m-1 (St/V) (Table 2).

For the scale-up of the reactor up to 1,250 L, the tube diameter was increased from 0.0378 to 0.050 m, to improve the volume/surface ratio while keeping the surface constant. Based on environmental conditions prevailing in the Atacama Desert, two modifications were made to the reactor. Firstly, the exposure angle of the spiral surface was modified to decrease exposure to the photon flow and to avoid photoinhibition. Secondly, the photobioreactor height was extended to increase the St/V ratio. The challenge is to increase the light interception to enlarge light exposition of the cells and then larger microalgae production [2, 28] (Figure 1).

The developed Fibonacci-type reactor consists of three main parts – the photoactive panel, the drive system and the gas exchange tank. The photoactive panel is made up of a continuous polyvinyl chloride transparent pipe, resistant to ultraviolet light, pH ranges from 3 to 14, temperature from 0 to 45º C and salinity from 5 to 300 g·L-1. The pipe arrangement is spiral or helical in shape, which decreases its perimeter towards the top, forming a truncated half-cone. It has a surface light/volume ratio of 77 m-1 (Figure 2). The base of the truncated half-cone is the section with the largest perimeter, and it is where the spiral starts; it is also where the CO2 inlet is located. The upward flow of the injected CO2 guarantees maximal efficiency in terms of its absorption by the microalgae. At the end of the spiral is the impeller system, which uses an "air-lift" system with air supplied by an S-41 "Blower" (Sweetwater Inc. USA). In this case, three pipe spirals were used to decrease the tube length, reducing the speed due to head losses and friction as well as minimising the distance the culture travels in each of the three pipes. Moreover, the volume that has to be mixed by the air-lift is smaller in each pipe thus allowing an increased culture flow rate. Finally, the interior 300 L gas exchange tank is arranged in a tower configuration over the photobioreactor. CO2 and air flow rates provided were 0.001 and 0.01 v/v/min respectively.

In experiments performed at laboratory conditions using the small scale reactor (6 L), it was illuminated at 175 μE·m-2·s-1, the pH was controlled by on-demand injection of CO2 to maintain the pH in the range between 7.5-8.0, while the temperature remained at 30-35 °C, and the dissolved oxygen concentration was not controlled since the spiral design allows the values to be kept below 150%Sat.

In experiments performed at outdoor conditions the large reactor (1,250 L) was in Pampa del Tamarugal. The reactor was installed and oriented to the north in the facilities belonging to the GATTAVARA SACI company (La Tirana, Iquique, Chile). Daily recordings were taken of the biological, physical and chemical variables. In this reactor the physical and chemical variables were recorded at the end of the upward spiral where the gas exchange tank is located. The controlled variables recorded were pH (1115001 4-STAR, Thermo Orion, UK), temperature, dissolved oxygen and the oxygen saturation percentage (55, YSI, USA). Measurements were taken at different times of the daylight phases: at 09:00 h, 13:00 h and 18:00 h. In addition, environmental variables such as temperature, humidity and irradiance were measured (Monitor-800027, Sper Scientific, USA; Radiometer 840029, Sper Scientific, USA).

## Laboratory measurements

The biological parameters of the cultures were daily measured. The cultures were observed at optical microscope to evaluate cells appearance. The dry weight content was determined by taking 30 ml sample and filtering it through a pre-combusted (550 °C, 2 h) and pre-weighed glass fibre filter (0.45 μm, Whatman), then it being washed with ammonium 0.5 M, dried at 105 °C and allowed to cool in a desiccator before being reweighed. Absorbance of the culture was also measured using a spectrophotometer (SP 830 Plus, Metertech, Taiwan) in 750 nm. The cell density (cellules/ml) was recorded daily for each sample; the cells were counted in a Neubauer chamber (0.1 mm depth).

## Average irradiance and specific growth rate

To determine the average irradiance inside the culture the simplified Equation 1 [29] was used. According to this equation the average irradiance is a function of the incident radiation (Io) on the photobioreactor surface and the attenuation of the light by the culture, it being a function of biomass concentration (Cb), pipe radius (R) and light attenuation coefficient (Ka).

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| --- | --- |
|  | Equation 1 |

To estimate the specific growth rate (μ) the Equation 2 was used [30]. This incorporates the light availability (Io), the illuminated surface of the photobioreactor, the extinction factor of the culture (Ɛ), the biomass concentration (Cb), the maximal growth rate (μmax) and the illumination to volume ratio (St/V).

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|  | Equation 2 |

## Software

Data were processed and analysed using Microsoft Excel 2016. The photobioreactor’s 3-D structure and modelling was developed using SolidWorks; this software was also used to determine the solar light availability on the reactor surface.

Results and Discussion

## Selection of culture medium

To develop a culture medium based on agricultural fertilizers instead of pure chemicals, up to four different media were formulated, the performance of green cells of *Dunaliella salina* on them being evaluated (Figure 3). Data shows as the performance of *Dunaliella salina* was maximal when using KSP medium, compared to the others (control SM, KSP MOD and BRIASCU). Biomass concentration at the end of batch culture was maximal, up to 0.84 g·L-1, when using KSP medium, followed by SM (control) with 0.77 g·L-1, KSP MOD with 0.69 g·L-1 and BRIASCU with 0.66 g·L-1. Nitrogen supply is essential for microalgal growth, especially the utilization of natural sources being recommendable to improve the sustainability of the process. In this case only KNO3 of natural origin was used. The KSP medium finally selected contains the larger concentration of nitrate. Also, this medium contains the larger concentration of phosphate. However, it does not contain bicarbonate or iron, despite that allowing to maximize the performance of green cells of *Dunaliella salina*.

To analyse the performance of *Dunaliella salina* in the Fibonacci type photobioreactor, an experiment was performed at laboratory conditions using the small-scale reactor. On this experiment the irradiance on the reactor surface was 106 μE·m-2·s-1, whereas the temperature on the laboratory was keep at 32 ºC. The temperature of the culture was 32±1 ºC while the pH was maintained at 8.0±0.1 by on-demand injection of CO2 (Figure 4A). No dissolved oxygen was accumulated inside the reactor, values below 120 %Sat. being measured.

These figures allow to confirm than the Fibonacci-type photobioreactor configuration allows the efficient control of the culture parameters into the culture. The temperature was the same as ambient confirming the high capacity of this design to dissipate heat to the ambient, thus compensating heat received from the light sources. The upward flow of the injected CO2 facilitates its absorption as there is no accumulation of CO2 bubbles in the spiral tube, pH variations being minimal during the complete batch culture, both at low and high biomass concentrations. Furthermore, no bubbles at the end of the pipe were observed, thus no CO2 is lost to the atmosphere. This fact allows to achieve an adequate control of the pH value and to reduce the production cost by maximizing the efficiency of CO2 utilization. The control of pH is crucial to maximize the performance of any microalgae culture and it also prevents cell damage [31]. Another characteristic of this photobioreactor is its efficient dissolved oxygen removal capacity. This is generally a relevant problem in tubular photobioreactors when the level exceeds 200% Sat., but also in open raceways [32–34]. In the Fibonacci-type reactor the oxygen degassing was sufficient to keep the maximum daily dissolved oxygen concentration below 120% Sat. This was due to the upward flow and the aeration in the degassing tank (Figure 4B**Error! Reference source not found.**). On the basis of oxygen accumulation into the loop an oxygen production rate up to 6.7 mgO2/L·h was estimated, then taking into account the driving force for oxygen desorption, it being equal to dissolve oxygen concentration into the liquid phase minus that in equilibrium with air, the volumetric mass transfer coefficient into the reactor was calculated as 5.4 1/h. This value is double than previously determined in tubular photobioreactors of small tube diameter, and close to ten times higher than reported for raceway reactors [33–35].

Concerning biomass concentration and productivity, data shows as *Dunaliella salina* achieves a maximum biomass concentration of 0.96 g·L-1 at the end of the batch culture, while the maximum biomass productivity achieved was 0.12 g·L-1·day-1 and 2.41 g/m2day on day 4th (Figure 4C). This biomass concentration is double that previously obtained in a raceway reactor (0.3 g·L-1) belonging to the Norbiotech company (now called GATTAVARA SACI). Anyway, the biomass productivity in the Fibonacci-type reactor at laboratory scale was limited by the low irradiance on the reactor surface being provided (175 μE·m2·s-1). To confirm this light limitation effect the average irradiance inside the culture was calculated (Equation 1) [29]. Data shows as the average irradiance inside the reactor decreased from 130 to 80 μE·m-2·s-1 during the batch culture because of biomass concentration increase. Due to reduction of average irradiance, the specific growth rate reduces from 0.16-0.04 day-1 during the batch culture, thus confirming the existence of light limiting conditions (Figure 4D).

## Scale-up of Fibonacci-type photobioreactor

Success of scale-up processes is largely a function of the right selection of characteristics parameters used. The objective of scale-up must be to achieve a larger size at the same time than providing the same culture conditions at small scale. Guidelines for optimal tubular photobioreactor design includes: (i) uniform culture illumination [36]; (ii) low dark to illuminated culture volume ratio [37]; (iii) low O2 partial pressure [18, 38]; (iv) high illuminated surface to volume ratio (St/V) [39]; (v) high ratio between the illuminated area and the ground area occupied by the reactor (St/S)[40, 41]; (vi) vertical orientation [42]; (vii) reduced mixing time [43]; and (viii) turbulent mixing.

Considering the above guidelines, the photobioreactor was scaled up with the main objective being to avoid microalgal photoinhibition. For this, the exposure angle of the surface that forms the spiral tube was increased by ten degrees with respect to the previous design [22]. This makes possible to increase the photobioreactor height, to increase the tube diameter to 0.0252 m and the volume of the photobioreactor to 1.25 m3. The height increase also provides a greater illuminated area with respect to the surface occupied, obtaining an St/V ratio of 77 m-1. This relationship is basic for enhancement of light capture capacity, the most important variable in biomass production, and the one used to compare photobioreactor designs (Table 2) [28].

Making the photobioreactor design closer to the natural architecture of trees, would offer us light exposure efficient strategies for diluting the flow of photons on the photosynthetic surface of the photobioreactor [26, 36]. Thus, the Fibonacci-type photobioreactor is inspired into the frond spiralling growth strategy of the trees, it is allowing the dilution of the light. The best example is *Prosopis tamarugo Phil*, a tree that is very well adapted to the extreme conditions of the Atacama Desert. Associated with this concept, if the light intensity is above the saturation level it can be not efficiently utilized, moreover at irradiances larger than the inhibition irradiance the excess of light inhibit the microalgal growth [10, 17, 44]. The irradiance on the reactor surface, in addition to the illuminated surface area, the occupied area and the reactor volume are the most important factors when comparing photobioreactors, the last ones being expressed usually as the volume to surface ratio, both occupied or exposed to light [28]. In the Fibonacci-type reactor developed the V/S ratio is 20 L m-2, much lower than values already reported for other closed photobioreactors designs as flat-panel and tubular, which range from 50-150 L m-2 [45]. Concerning open reactors, this V/S ratio is also much lower than regular value of 200 L·m-2 found in raceway reactors when operating at 0.2 m water depth, but it is the same to that reported for thin-layer reactors then operating at 0.02 m water depth (20 L·m-2), although on this thin-layer reactors V/S ratio up to 5 L·m-2 have been reported when operating at 0.005 m water depth [46]. It is important to note that in general a higher biomass productivity per land surface occupied is reported when the V/S ratio increases [46].

The microalgae biomass productivity on any type of reactor is largely a function of solar radiation intercepted, it is a function of the surface occupied by the photobioreactor and its position. To evaluate the adequacy of whatever photobioreactor design, it is interesting to compare the light interception capacity with respect to solar energy intercepted by a horizontal surface. In the case of raceway reactors, the solar radiation intercepted is limited by its surface. In the case of tubular photobioreactors the solar radiation interception is limited by the height of the loop and spatial distribution of the tubes. In the case of the Fibonacci-type photobioreactor, radiation intercepted was determined by using SolidWorks software (Figure 5). Results shown as the solar radiation intercepted by the photobioreactor surface at large scale (1,250 L) varies during the day according to the position of the sun, achieving maximum solar radiation when it is oriented towards the sun. The maximum radiation intercepted by the Fibonacci-type photobioreactor is 1.8-times that captured received by an horizontal surface, a previous value of 1.4-times being reported [22] (Figure 5).

Analysis of annual variation of solar radiation intercepted shows as in winter and autumn seasons, there is a larger variability in the capture of solar radiation on the different parts of the reactor (variation of angle with respect to the north), unlike in summer and spring the light interception is more homogeneous (Figure 6A). To provide homogeneous irradiance on the reactor surface is highly relevant to minimize the photoinhibition effect when excess of solar radiation arrives to the reactor surface. The proposed design allows to capture more sunlight than the horizontal surface regardless of the season, values ranging from 1.6 to 1.9 times larger (Figure 6B). In the previous design, light interception improvement was up to 1.2 times that received at ground level [22]. The larger solar radiation interception capability of the new design is due to the increased angles that modifies the reactor surface. The value here reported for the large scale reactor is much higher than previously reported for flat-panel reactors (1.2 times), it being also largely a function of orientation and geographical location [47].

In addition to solar radiation interception, the capacity of heat removal to maintain moderate temperatures is also critical in any kind of photobioreactor design. Overheating is one of the biggest drawbacks in both horizontal and vertical tubular photobioreactors since the culture temperature is up to 10-30 °C higher than the ambient temperature. Therefore, cooling and heating systems must be incorporated into them [48–50]. It is important to keep in mind the location of the experiments performed. Pampa del Tamarugal (in the Atacama Desert) experiences average maximum and minimum temperatures in winter of 32 °C and 1 °C, respectively. At these conditions, the temperature of the culture on the Fibonacci-type photobioreactor at different times of the day was evaluated. At 9:00 am, the reactor has an average temperature of 17.7 °C, showing a temperature distribution more centred on the average. Outdoors, the average temperature is 18.2 °C with a more heterogeneous distribution. The difference between the temperatures therefore is 0.5 °C (Figure 7). At 1:00 pm, when maximum solar radiation occurs, the average photobioreactor temperature is 28.0 ° C while outdoors it is 23.5 °C - the photobioreactor temperature distribution is now wider, 4.5 °C above the outdoor temperature (Figure 7). At dusk (6:00 pm), the difference in terms of averages between the reactor and the outdoor temperature is significantly reduced, to approximately 3.1 °C (Figure 7).

## Evaluation of performance of large-scale Fibonacci-type photobioreactor

To evaluate the performance of the large-scale Fibonacci-type photobioreactor for the production of Dunaliella salina, it was inoculated and operated in semi continuous mode. The reactor was located at Pampa del Tamarugal (in the Atacama Desert). During the experiment the average solar irradiance in the light period ranged from 995 to 600 μE·m-2·s-1 with only one cloudy day (Figure 8A). Inside the photobioreactor, the average culture temperature ranged from 18.2 to 22.5 °C (Figure 8A). As explained above, overheating does not take place despite the high irradiation by the excellent heat removal capacity on the proposed design. The pH was maintained in the 7.5 to 8.5 range with an average of 8.0 (Figure 8B). The dissolved oxygen concentration was always kept below 120 %Sat. Equal than in the small scale reactor, on the basis of oxygen accumulation into the loop the oxygen production rate was measured, maximal values up to 9.2 mgO2/L·h being estimated, then taking into account the driving force for oxygen desorption the volumetric mass transfer coefficient into the reactor was calculated as 9.4 h-1. This value is double than previously determined for the small-scale reactor of 5.4 h-1 due to the higher turbulence on the larger scale reactor. The adequacy of proposed design to control temperature, pH and dissolved oxygen concentration inside the culture allows to improve the performance of the culture and to avoid adverse effects such as photoinhibition and photo-oxidation phenomena [51]. The proposed design allows to increase the solar radiation interception it increases the photosynthesis rate then the CO2 demand and oxygen production rates. Despite this high demand the proposed Fibonacci-type reactor was able to control both variables, such as pH and dissolved oxygen, thus avoiding adverse culture conditions for the cells.

Concerning biomass concentration, a maximum value of 0.96 g·L-1 was achieved when operating in semi-continuous mode, harvesting 30% of the total volume once per week (Figure 8C). Biomass concentration achieved was three-times larger than that achieved with the same strain in a raceway. Regarding biomass productivity, considering the biomass collected when harvesting the reactor and the time between dilutions overall values of 0.065, 0. 43 and 0.045 g·L-1·day-1 were measured for each one of the three steady states achieved. However, analysing the variation of biomass concentration on each day daily values were calculated, values ranging from 0.01 to 0.12 g·L-1·day-1 being obtained (Figure 8C). It was observed as the maximal biomass productivity was obtained at biomass concentrations ranging from 0.7 to 0.8 g·L-1, above and below this value the biomass productivity decreased. Biomass productivity values up to 0.08 g·L-1·day-1 have been reported for *Dunaliella salina* produced in outdoors tubular photobioreactor in semi-continuous mode [52]. On raceway reactors, values up to 0.3 g·L-1·day-1 have been reported [49]. These values are much lower than those reported for other microalgae strains because *Dunaliella salina* is a slow growing algae.

Data confirm that it was possible to operate the photobioreactor in continuous mode, achieving stable quasi-steady states. Biomass productivity achieved was limited by the light availability by the cells, in spite of the large interception of solar radiation by the proposed design the cultures being mainly photo limited. Thus, the average irradiance daily values ranged from 100 to 200 μE m-2·s-1 during the semi-continuous operation of the reactor, daily values of specific growth rate ranging from 0.02 to 0.17 day-1 (Figure 8D). Maximal productivity was obtained at average irradiance value of 150 μE m-2·s-1 thus indicating that this is the constant irradiance value for this strain, also the maximal growth rate achieved on the reactor being of 0.17 day-1. These values are much lower than those reported for other microalgae strains of fast growth such as *Scenedesmus* or T-ISO, for which constant irradiance values lower than 100 μE m-2·s-1 and specific maximum growth rates up to 1 day-1 are reported [53, 54]. On this way, to demonstrate the high performance of Fibonacci-type reactor developed to evaluate it with fast growing strains such as reported will be carried out.

One of the most relevant variables for the scaling of the Fibonacci type photobioreactor from 330 L to 1250 L, is related to the illuminated surface of the photobioreactor since it is one of the most important factors in the production of biomass. In this sense, the illuminated surface was increased from 39.6 (330 L) to 96.7 (1250 L) on the same occupied surface. This increase in the illuminated surface, undoubtedly allows to increase the volume of the new design of photobioreactor, allowing to have more volume in the same surface, passing from a relation Land occupied / volume 191 to 50 m-1.

A production cost has been estimated for the cultivation of *Dunaliella salina* in this type of photobioreactor. With the production data obtained in the experimentation, a plant with 50 photobioreactors has been modelled with an annual production of 38 tons per year in an area of 0.56 hectares and an investment of US$ 788000. The cost of production is US$ 20 per kg of dry biomass, where CO2 and staff are the items with the highest percentage participation in the cost, respectively 31% and 27% ( Figure 9).

Conclusions

In this work, a Fibonacci-type photobioreactor was scaled up under the extreme conditions of the Atacama Desert. The *Dunaliella salina* microalgae strain was tested while the reactor was evaluated on two scales, under indoor and outdoor conditions. The results show that the Fibonacci-type photobioreactor allows to capture 1.6-times more solar radiation than the horizontal surface, while providing optimal conditions for *Dunaliella salina* cultivation without the need for cooling, it also allowing maximal efficiency of CO2 utilization and to avoid excessive dissolved oxygen accumulation. Thus, operating in semi-continuous mode biomass concentration up to 0.96 g·L-1, biomass productivity up to 0.12 g ·L-1·day or 2.41 g/m2day, and maximal specific growth rates of 0.17 day-1 were measured. These figures demonstrate that the Fibonacci-type photobioreactor can be used to produce green cells of *Dunaliella salina* at commercial scale.

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Table 1. Culture medium for experimental growth of *Dunaliella salina* in the laboratory, in a 6 L Fibonacci photobioreactor.

|  |  |
| --- | --- |
| Medium / Composition | g·L-1 |
| **KSP** |  |
| KNO3 agricultural origin | 0.5 |
| Triple Super Phosphate | 0.05 |
| Sea salt | 150 |
| Sol. EDTA-Fe Johnson (mL/L) | 10 |
|  |  |
| **KSP Mod** |  |
| KNO3 agricultural origin | 0.3 |
| Sea salt | 150 |
| NaHCO3 | 3 |
| Sol. FDA (mL/L) | 0.3 |
| Sol. EDTA-Fe (mL/L) | 0.1 |
|  |  |
| **SM** |  |
| KNO3 agricultural origin | 0.06 |
| Triple Super Phosphate | 0.02 |
| Sea salt | 150 |
| NaHCO3 | 0.5 |
| Sol. EDTA-Fe Johnson (mL/L) | 10 |
|  |  |
| **BRIASCU Mod** |  |
| KNO3 agricultural origin | 0.05 |
| Triple Super Phosphate | 0.025 |
| Sea salt | 150 |
| NaHCO3 | 4.2 |
| FeSO4 | 0.025 |
| HCl (mL/L) | 1 |
| FeCl | 0.09 µM |
| Na2EDTA | 0.05 µM |

Table 2. Main parameters of indoor and outdoor photobioreactors utilized for the production of *Dunaliella salina*.



\*\* SOURCE [22].

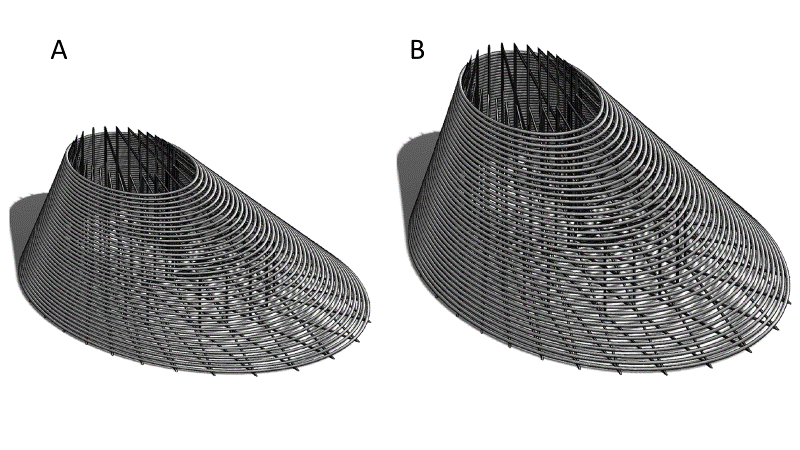


Figure 1. Scheme of Fibonacci-type reactor at small and large scale. A) 330 L reactor, B) Scale-up to 1,250 L reactor.

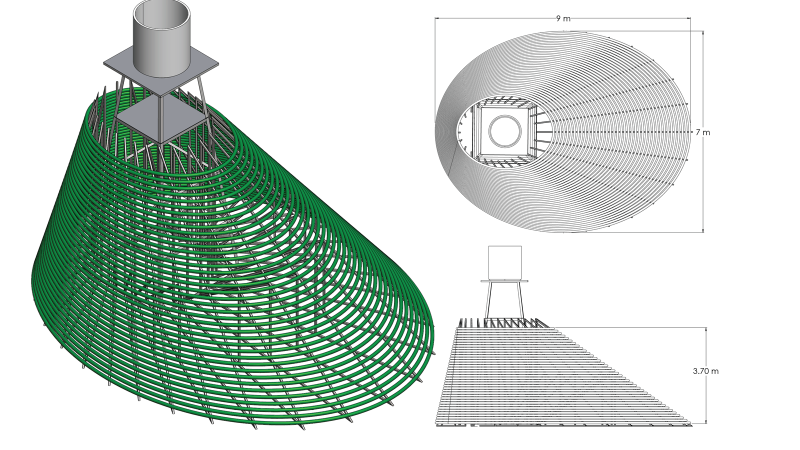


Figure 2. 3D representation of the Fibonacci Photobioreactor scaled to 1,250 L. It shows its tube support structure that makes up the spiral.

Figure 3. Average of biomass concentration values in time of *Dunaliella salina* cultures performed with different culture media on the small-scale Fibonacci-type reactor indoor (6 L).



Figure 4. Batch culture of *Dunaliella salina* in the small-scale Fibonacci-type photobioreactor 6 L at indoor conditions. A) Irradiance and temperature; B) pH and dissolved oxygen; C) Biomass concentration and productivity; D) Average irradiance and growth rate (2008, September, Atacama Desert).



Figure 5. Light intercepted by the surface of the Fibonacci-type photobioreactor scaled-up to 1,250 L at different times of the day (8:00, 10:00, 12:00, 14:00 and 16:00). Performed using SolidWorks Flow Simulation (2008, September, Atacama Desert).

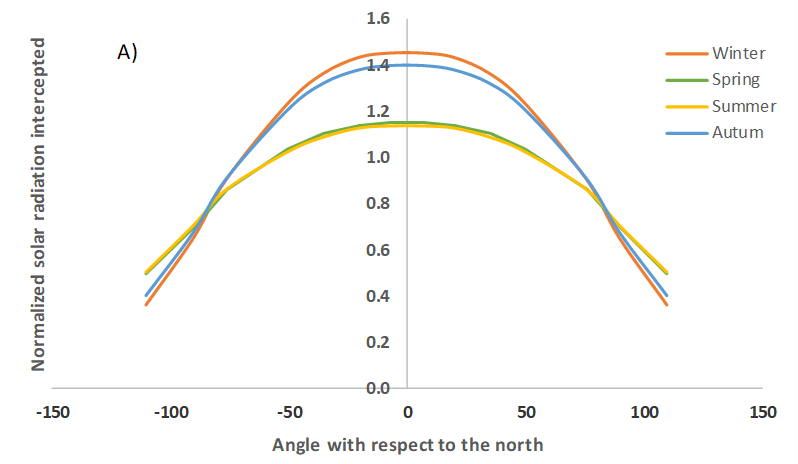




Figure 6. A) Normalized irradiance on the surface of reactor with respect to that received on the horizontal surface on different seasons; B) Total solar radiation intercepted respect to the horizontal surface, and the normalized value (2008, September, Atacama Desert).



Figure 7. Evolution of the temperature over three hours inside the photobioreactor and in the environment (2008, September, Atacama Desert).



Figure 8. Semi-continuous culture of *Dunaliella salina* in Fibonacci-type photobioreactor scaled-up to 1,250 L at outdoor conditions. A) Irradiance and temperature; B) pH and dissolved oxygen; C) Biomass concentration and productivity; D) Average irradiance and growth rate (2008, September, Atacama Desert).

Figure 9. Unit cost to produce *Dunaliella salina*, in Fibonacci photobioreactor.