Nuevo diseño de fotobiorreactor tubular basado en la configuración de Fibonacci

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Dedicatoria

A Dios, por su infinita misericordia y permitirme leer el libro de la naturaleza.

A mis padres y hermanos, con profundo amor y respeto.

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"El hombre no crea: descubre y toma ese descubrimiento. Los que buscan las leyes de la naturaleza para formar nuevas obras, colaboran con el Creador […] Por eso la originalidad consiste en volver al origen"

Antoni Gaudi (1852-1926)

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Dedication

To God, for his infinite mercy and allowing me to read the book of nature.

To my parents and siblings, with deep love and respect.

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Antoni Gaudi (1852-1926)

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Resumen

Para el 2050 el mundo necesitará satisfacer la necesidades de nueve mil millones de personas. Esto sin duda contribuirá a seguir amentando la demanda por recursos naturales, de alimentos y suministros energéticos, causando aumento de contaminación y favoreciendo cambio climático. La satisfacción de estas necesidades deberá ser bajo un enfoque de desarrollo sustentable, es decir, satisfacer las necesidades presentes sin comprometer las generaciones futuras, puesto que seguir el patrón de consumo actual se necesitarían más planetas tierra, cosa que no es posible. En este contexto, las microalgas podrían proporcionar una gama interesante de productos, puesto que, son una fuente potencial de sustancias bioquímicas y nutricionales como proteínas, lípidos, carbohidratos, antioxidantes, pigmentos, con altas posibilidades económicas, con usos en la industria alimentaria para consumo humano y como aditivos para piensos, farmacéuticas y nutracéuticas.

Las microalgas convierten la energía solar en productos de almacenamiento de carbono, pudiéndose convertir en una alternativa de energía renovable sostenible, ya que tienen un alto potencial para producir grandes volúmenes de biomasa que, a su vez, se puede utilizar en la producción de diferentes biocombustibles. Además, ayudarían a mitigar la acumulación de CO_2 (Dióxido de carbono) en la atmosfera, ya que ellas necesitan de este para realizar la fotosíntesis. Por otra parte, son muy eficientes en el tratamiento de aguas residuales permitiendo reducir el consumo de agua, así como recuperar nutrientes de las aguas residuales, como el fósforo y los nitratos. Por todas estas razones, los procesos basados en el uso de microalgas están recibiendo un creciente interés dentro del sector industrial a nivel mundial.

Entonces el desafío es, industrializar la producción de microalgas, para ello es necesario contar con tecnologías eficientes para el cultivo de microalgas, vale decir fotobiorreactores. No obstante, Se ha realizado avances en esta materia pero quedan muchas tareas por realizar. Por tanto, la gran tarea pendiente es diseñar fotobiorreactores eficientes, siendo necesario capturar la mayor cantidad de luz, bien distribuida o diluida en la superficie del fotobiorreactor. La presente investigación hace su aporte al proponer un nuevo diseño de fotobiorreactor tubular inspirado en la geometría de Fibonacci, que permitió mejorar la disponibilidad de luz y productividad del cultivo, con la posibilidad de ser escalado a un nivel industrial. Los resultados confirman que el diseño propuesto permite un aumento en la captación en la radiación solar interceptada en comparación con la recibida en una superficie horizontal, al tiempo que proporciona condiciones óptimas de cultivo para el crecimiento de microalga como temperatura moderada, pH adecuado y niveles aceptables de oxígeno disuelto, mejorando la productividad en el fotobiorreactor.

Esta tesis presenta los trabajos desarrollados para poner a punto y escalar un reactor tipo Fibonacci para la produccion de microalgas. En el primer trabajo se propone el nuevo diseño de fotobiorreactor tubular inspirado en la geometría de Fibonacci. Esto proviene de observar la geometría de los organismos fotosintéticos en su estado natural, donde el crecimiento de la planta forma giros, espirales o bobinas; patrones clasificados como helicoidales. Comienza con una pequeña unidad de 6 L se evaluó en interiores y una unidad más grande de 250 L en exteriores; ambas unidades mantuvieron el mismo concepto y un criterio de relación de diámetros de las elipses superior e inferior. Para probar el nuevo diseño, se utilizó la cepa *Arthrospira (Spirulina) platensis*. Los resultados muestran que el diseño propuesto permite mantener la temperatura, el pH y el oxígeno disuelto dentro de los rangos óptimos recomendados para la cepa seleccionada. En consecuencia, se evitó el sobrecalentamiento, incluso en condiciones exteriores, y la concentración de oxígeno disuelto se mantuvo por debajo del límite del 200% Sat. La modelización del crecimiento en ambos reactores demostró que el rendimiento de la célula era óptimo en condiciones interiores y exteriores, con tasas de crecimiento específicas máximas al aire libre que alcanzaban 0,8 1/día. Además, en condiciones exteriores, la eficiencia fotosintética alcanzó el 5,4%.

En el segundo trabjo, el fotobiorreactor tipo Fibonacci propuesto anteriormente se escala hasta 1200 L y se evalúa para producir *Dunaliella salina*. El reactor se amplió manteniendo una alta capacidad de intercepción de radiación solar de este tipo de reactor. Se evaluó el desempeño del reactor para la producción de células verdes de *Dunaliella salina* en las condiciones ambientales que prevalecen en el Desierto de Atacama. Los datos demostraron que el fotobiorreactor propuesto permite mantener la temperatura, el pH y la concentración de oxígeno disuelto dentro de los rangos óptimos recomendados para la cepa seleccionada. Tanto una mejor exposición a la radiación solar como la dilución del flujo fotónico evitan el uso de sistemas de refrigeración para evitar el sobrecalentamiento en condiciones exteriores. El sistema permite interceptar hasta un 60% más de radiación solar que la superficie horizontal. La concentración de biomasa alcanzó hasta 0,96 g L^{-1}, tres veces superior a la obtenida en un reactor de regatas en las mismas condiciones ambientales, mientras que la productividad fue de hasta 0,12 g L^{-1} $día^{-1}$ (2,41 g m^{-2} $día^{-1}$). Las tasas máximas específicas de crecimiento al aire libre alcanzaron hasta 0,17 $día^{-1}$.

Finalmente, en un tercer trabajo se escala hasta 2500 L del fotobiorreactor tipo Fibonacci para la producción de la microalga *Chlorella* sp. Los resultados muestran que este diseño de fotobiorreactor mantuvo el cultivo de *Chlorella* en su temperatura óptima, pH y niveles de oxígeno disuelto. A pesar de ser un reactor cerrado, las temperaturas se mantuvieron por debajo de los 30 ºC mientras que el oxígeno disuelto se mantuvo por debajo del 200 % Saturción. en condiciones exteriores en las que la radiación solar media diaria osciló entre 139 y 450 µE/m^2s. En estas condiciones, se alcanzó una tasa de

crecimiento específica máxima de 0,31 día^{-1}, una concentración de biomasa de 1,9 g/L y una productividad volumétrica de biomasa de 0,37 g/L día.

El desarrollo de la biotecnología de microalgas a escala industrial requiere fotobiorreactores más productivos y eficaces capaces de maximizar la productividad de la biomasa en condiciones de alta luz. El fotobiorreactor tubular tipo Fibonacci que aquí se presenta ofrece avances sustanciales en comparación con los fotobiorreactores tubulares clásicos, ya que ha demostrado ser flexible en su diseño geométrico y puede adaptar sus parámetros a las necesidades fotosintéticas de las microalgas para garantizar la máxima eficiencia, lo que lo convierte en una alternativa a los sistemas tubulares convencionales. Por último, se presenta la estrategia para determinar el diseño óptimo de los fotobiorreactores tipo Fibonacci.

Como ejemplo de aplicación de la tecnología propuesta se completa la presente tesis con los ensayos realizados sobre el uso de la biomasa de microalgas producida como complemento nutricional en piensos de ganadería, en concreto en piensos de gallinas. En dichos ensayos se ensayaron dietas enriquecidas con *Spirulina platensis* y *Haematococcus pluvialis* analizándose la mejora tanto en la productividad como en la calidad de los huevos producidos por las gallinas alimentadas con estos piensos. Los resultados mostraron una mejora significativa tanto en la cantidad como calidad de huevos producidos cuando se incorporan microalgas al pienso, confirmándose así el interés en el desarrollo de nuevos estudios que soporten el desarrollo de este tipo de aplicaciones de la biomasa de microalgas, incluidas las mejoras en su producción a mayor escala utilizando la tecnología desarrollada en esta tesis.

Summary

By 2050, the world will need to meet the needs of nine billion people. This will undoubtedly contribute to further increasing the demand for natural resources, food and energy supplies, causing an increase in pollution and favouring climate change. To accomplish these needs must be based on a sustainable development approach, that is, satisfying present needs without compromising future generations since the current pattern of consumption would require more planet Earth, which is not possible. In this context, microalgae could provide an interesting range of products, since they are a potential source of biochemical and nutritional substances such as proteins, lipids, carbohydrates, antioxidants, and pigments, with high economic possibilities, with uses in the food industry for human consumption and as additives for animal feed, pharmaceuticals and nutraceuticals.

Microalgae convert solar energy into carbon storage products, which could become a sustainable renewable energy alternative, as they have a high potential to produce large volumes of biomass that in turn, can be used for the production of different biofuels. In addition, they would mitigate the accumulation of Co_2 (carbon dioxide) in the atmosphere since they need carbon dioxide for photosynthesis. Moreover, they are very efficient in wastewater treatment, allowing to reduce water consumption, as well as recover nutrients from wastewater, such as phosphorus and nitrates. For all these reasons, processes based on the application of microalgae are receiving increasing interest within the industrial sector worldwide.

Therefore, the challenge is to generate technology to industrialize the production of microalgae, for which it is necessary to have efficient technologies, i.e. photobioreactors. Relevant advances have been performed in this area, but many tasks remain to be done. Therefore, the great pending task is to design efficient photobioreactors, being necessary to capture as much light as possible, either distributed or diluted on the surface of the photobioreactor. The present research focuses on this topic by proposing a new photobioreactor desing inspired by the Fibonacci geometry, which allows optimizing light availability and productivity, with the possibility of being scaled up to an industrial level. The results show that the proposed design increases the solar radiation intercepted, compared to that received on a horizontal surface, providing optimal conditions for the growth of microalgae, while maintaining a moderate temperature, adequate pH and low dissolved oxygen concentration, improving the productivity of microalgae cultivation.

This PhD thesis summarized the work performed to develop and scale up the Fibonacci type photobioreactor for the production of microalgae. In the first work, the new design of the tubular photobioreactor inspired into the Fibonacci geometry is showed. This comes from observing the geometry of the photosynthetic organisms in their natural state, where the plant growth forms turn,

spirals or coils; patterns classified as helical. A small 6 L unit was evaluated indoors and a larger 250 L unit was evaluated outdoors; both units maintained the same concept and were developed using as the criterion to maintain the ratio of diameters of the upper and lower ellipses. The new photobioreactor design was tested with the *Arthrospira (Spirulina) platensis* strain. The results show that it is possible to maintain the temperature, pH and dissolved oxygen in the optimal ranges recommended for the cultivation of the strain. That is, there was no overheating of the photobioreactor, and the dissolved oxygen concentration remained below the of 200% Saturation limit. The growth model applied in both reactors showed that maximum specific outdoor growth rates reached 0.8 1/day, and photosynthetic efficiency reached 5.4%.

In the second article, the Fibonacci-type photobioreactor is scaled up to 1200 L and the microalgae strain *Dunaliella salina* is used. The reactor was scaled up maintaining the design parameters that allow greater interception of solar radiation around its surface. The productivity of the photobioreactor was evaluated through the production of the Dunaliella salina strain under extreme environmental conditions, such as those of the Atacama Desert. The results show that the fibonacci type photobioreactor allows maintaining the temperature, pH and dissolved oxygen concentration within the optimal ranges for Dunaliella salina cultivation. The improved exposure to solar radiation in this photobioreactor, such as the dilution of the photonic flux, and the low thermal conductivity of the photobioreactor tube (PVC), avoid the use of cooling systems in outdoor conditions to maintain the temperature. The proposed photobioreactor can intercept up to 60% more solar radiation than the horizontal surface. Reaching a biomass concentration of 0.96 g L^{-1}, three times higher than the result obtained in a receway for dunaliella cultivation at commercial level, under the same extreme environmental conditions, while the productivity reached 0.12 g L^{-1} day^{-1} (2.41 g m^{-2} day^{-1}). The specific growth rate reached up to 0.17 day^{-1}.

Finally, the Fibonacci photobioreactor was scaled up to 2500 L and cultured in it of *Chlorella* sp. The results show that this photobioreactor design maintains the culture conditions at its optimal pH, temperature, and dissolved oxygen levels. Despite being a closed reactor, the temperature was kept below 30ºC while the dissolved oxygen was maintained below 200 % Sat. under outdoor conditions at which the mean daily solar radiation ranged from 139 to 450 µE/m^2s. Under these conditions, a maximum specific growth rate of 0.31 day^{-1}, a biomass concentration of 1.9 g/L, and volumetric biomass productivity of 0.37 g/L day were achieved.

Biotechnological development for the industrial production of microalgae requires new photobioreactors capable of maximizing productivity and biomass production under high light conditions.. The Fibonacci-type tubular photobioreactor presented here offers substantial advances

compared to classic tubular photobioreactors, as it has proven to be flexible in its geometric design and can adapt its parameters to the photosynthetic needs of the microalgae to ensure maximum efficiency, thus making it an alternative to conventional tubular systems. Lastly, the strategy for determining the optimal design of Fibonacci-type photobioreactors is presented.

As an example of the application of the proposed technology, this thesis is completed with the tests carried out on the use of microalgae biomass produced as a nutritional supplement in livestock feed, specifically in chicken feed. In these trials, diets enriched with *Spirulina platensis* and *Haematococcus pluvialis* were tested, to analyze the improvement of the eggs produced by hens supplemented with these microalgae in terms of egg quality and egg laying. The results showed a significant improvement in egg production and egg quality when microalgae are incorporated into the feed, thus confirming the interest in the development of new studies that support the development of this type of applications of microalgae biomass, including improvements in its large-scale production using the technology developed in this thesis.

INDEX

1. HYPOTHESIS AND OBJECTIVE

1.1. HYPOTHESIS

"The great book, always open and always worth Reading is Nature"

Antoni Gaudi (1852-1926)

Open reactors have been developed since the 1950s to produce microalgae, due to their simplicity and low construction cost. These advantages make them useful for biomass production on an industrial scale (Y Chisti, 2007). However, open reactors are greatly affected by environmental conditions that hinder biomass production under outdoor conditions with large variations in light and temperature that occur during the solar cycle and throughout the year. In addition, variations in dissolved oxygen and culture pH can affect the performance of these reactors. In this sense, the accumulation of dissolved oxygen reduces the rate of photosynthesis in microalgae cultures when high biomass productivity is achieved (Molina et al., 2001). However, raceways have been improved over time in terms of reactor engineering, biomass production capacity, chemical, physical and biological modelling to account for temporal and spatial variations in these types of systems, and control strategies to improve reactor efficiency. (Fernández et al., 2012)(Fernández et al., 2014) (Barceló-Villalobos, et al., 2019).

Traditionally microalgae production is mainly performed in open raceway reactors, but in the world it has started to be used tubular photobioreactors. These reactors permit almost any microalgal strain to be produced, and the microalgae to be cultivated under more controlled conditions, allowing a higher quality of biomass and a lower risk of contamination. However, tubular reactors have certain drawbacks related to temperature control, optimal CO_2 supply and oxygen removal, all dependent on light capture by the reactor (Rubio Camacho et al., 1999)(Xing Z, Zong X, 2013). It has been systematically reported that maintaining optimal temperatures in tubular photobioreactors improves the growth of microalgae strains, being necessary to know the tolerance limits, since most microalgae strains range between 10 a 40 ºC (Bitog et al., 2011)(Ras et al., 2013). Regarding the pH level, carbon dioxide is usually injected on-demand to control it and to provide inorganic carbon, thus avoiding carbon limitation. The pH limits that most strains can tolerate range from 4 to 9, except for cyanobacteria, which can tolerate up to pH 11 (T. A. Costache et al., 2013)(Ippoliti et al., 2016)(Bao et al., 2012). In relation to the amount of oxygen in the culture, it has been shown that an excessive accumulation of dissolved oxygen above 20 mg/L significantly reduces the photosynthetic rate of most strains of microalgae; higher than this value, photorespiration may even occur (T. A. Costache et al., 2013)(Ippoliti et al., 2016)(Huang et al., 2017)(Reinemann & Timmons, 1989). Altogether these parameters are

modified in tubular photobioreactors as a function of the reactor design and the solar irradiance intercepted; thus, both the heat balance and the microalgae cell performance are dependent on the light captured by the tubes comprising the solar collector.

Therefore, a wide range of tubular photobioreactor configurations have been proposed over time, such as the helical ones (Hall et al., 2003), double loop (Torzillo et al., 1993) and horizontally or vertically arranged wall photobioreactors (Bosma et al., 2014). The most commonly employed at the commercial scale is the wall configuration, which is currently being used mainly to produce *Chlorella* and *Haematococcus* species (Chen et al., 2016). However, there is still a long way to go in the design of tubular photobioreactors, since they must be optimized to maintain microalgae cultures at optimal conditions of temperature, pH and dissolved oxygen, with minimum energy consumption of energy and contamination risk (Xing Z, Zong X, 2013)(Grobbelaar, 2010).

In this thesis, a new photobioreactor inspired by Fibonacci's geometry is proposed and developed. This comes from observing the geometry of the photosynthetic organisms in their natural state, where e.g. plant growth forms can be, spirals or coils; patterns classified as helical (Smyth, 2016)(Steeves & Sussex, 1989). Understandably, the large number of logarithmic spiral designs are abundant in nature, as mathematician Leonardo Fibonacci discovered.. For maximum productivity in a tubular photobioreactor, 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 (Díaz et al., 2019).

Of vital importance, is the development of more efficient photobioreactors will facilitate the improvement of both the enlargement of microalgae production capacity and the reduction of production costs. This is the way to also enlarge the portfolio on which microalgae can be used, further than current applications mainly related to nutraceuticals and foods. Thus, it has been largely reported that microalgae can be also utilized to improve the production of foods in the primary sector such as agriculture and livestock production feed (Vigani et al., 2015). In this sense, the utilization of microalgae biomass as feed for the production of eggs by poultry was also studied.

The hypotheses to be resolved by this thesis are presented below:

a) **Hypothesis 1.** The new Fibonacci photobioreactor design allows the cultivation of microalgae.

b) **Hypothesis 2.** It is possible to scale up the Fibonacci-type photobioreactor using different strains of microalgae (*Arthrospira (Spirulina) platensis, Dunaliella salina* and *Chlorella sp.).*

c) **Hypothesis 3.** The Fibonacci-type photobioreactor is optimized to the requirements of photosynthesis rate and productivity.

d) **Hypothesis 4.** The addition of microalgae *Spirulina Platensis* and *Haematococcus Pluvialis* in the feed of laying hens (Hy line Brown) improves production performance, and egg quality.

1.2. OBJECTIVE

The general objective of this work is to propose a new design of tubular photobioreactor inspired by Fibonacci's geometry, allowing optimization of light availability and productivity of microalgae biomass. With the possibility of scaling it up to the industrial level. To achieve this general objective, the following specific objectives are proposed:

1. To propose a new design of a tubular photobioreactor created on the Fibonacci geometry, a spiral captured from nature.
2. Scale up the photobioreactor to different sizes, evaluating its performance with three strains of microalgae (*Arthrospira (Spirulina) platensis, Dunaliella salina and Chlorella sp*.).
3. Assessing the irradiance, pH, temperature and dissolved oxygen on this reactor and finally the biomass concentration and productivity. To evaluate the performance of the Fibonacci-type photobioreactor for the production of different strains.
4. To optimize the design of Fibonacci's based reactor as a function of diameter and length of the tube, maintaining the volume and surface ratio, to estimate the optimal length of the tube that maximizes productivity for scale-up purposes.
5. Evaluation of the effect of the addition of different concentrations of microalgae *Haematococcus Pluvialis and Spirulina Platensis*, in the diet of laying hens in improving egg quality and product performance.

2. INTRODUCTION

2.1. Relevance of microalgae

To face the problems related to the increase in the world population, the rapid depletion of natural resources and climate change, new technologies capable to improve the production of foods and feed related materials are needed. Gro Harlem Bundlandt points out: “If 7 billion people were to consume as much energy and resources as we do in the West today, we would need 10 worlds, not one, to satisfy all our needs” (Brundland, 1987). It is estimated that the world's population will exceed 9 billion people by 2050. New consumption patterns threaten natural resources, food and energy security, causing pollution and climate change. Policymakers and investors are responding to this situation by supporting green technology and developing various regulatory and policy measures that move society in a more "sustainable" direction. (Pagett, 2018).

A large diversity of products could be obtained from microalgae, to be used in agriculture and/or the pharmaceutical industry (Pulz & Gross, 2004). Microalgae are a source potential of bio-active and nutritional substances as proteins, lipids, carbohydrates, antioxidants, and pigments, with high economic possibilities, with uses in the food industry for human consumption and as feed additives (Mobin & Alam, 2017)(Priyadarshani & Rath, 2012)(Vigani et al., 2015)(Sigamani et al., 2016), pharmaceutical and nutraceutical (Koller et al., 2014)(Raja et al., 2008). An example of this is the rapid development of microalgal biotechnology taking place in different countries such as France, the United States, China, Japan, Taiwan and Thailand. Most related to microalgae production processes for food and functional food markets; in particular microalgae used in bread, yoghurt and beverages, among others (Microalgae, Old Sustainable Food and Fashion Nutraceuticals, 2017).

Atmospheric accumulation of greenhouse gases (GHGs) and industrialization is considered the main cause of global climate change, with carbon dioxide (CO_2) and methane (CH_4) being the main contributors. CO_2 is considered the most important GHG, accounting for approximately 77% of total emissions, while CH_4 is emitted to a lesser extent (14%), but has a global warming potential 34 times greater than the CO_2 (Hoegh-Guldberg et al., 2019). Microalgae indicate alternative renewable sustainable energy sources as they have a high potential for producing large volumes of biomass which in turn can be used for the production of different third-generation biofuels on a large scale. Microalgae convert energy (solar) into carbon storage products, which leads to lipid accumulation. Advances in hybrid technologies, such as: biomass production, wastewater treatment, greenhouse gas mitigation for the production of core products as biofuels offer atmospheric pollution control such as the decrease of GHG (CO_2

fixation) combination waste-water treatment with microalgae growth (Maity et al., 2014)(Y. H. Wang et al., 2008). Wastewater treatment allows reducing water consumption as well as recovering nutrients from wastewater, such as phosphorus and nitrates. For all these reasons, the processes built on the use of microalgae are receiving a growing interest within the industrial sector worldwide (Yusuf Chisti, 2008) (Mata et al., 2010)(Luisa Gouveia, 2011)(Mehrabadi et al., 2015).

Photosynthetic organisms, cyanobacteria and microalgae, exhibit many physiological properties that are ideal for the production of numerous biotechnological products (Lü et al., 2011). These microorganisms generally live in complex habitats, where they can generally survive in extreme conditions environmental conditions of temperature, salinity, water insufficiency and exposure to UV radiation (Abed et al., 2006)(Seckbach, 2007). In addition, cyanobacteria and microalgae can grow photoautotrophically, heterotrophically or mixotrophically depending on the type of carbon source available (Chojnacka y Noworyta 2004). In addition, One of the characteristics of microalgae and cyanobacteria is that they can grow photoautotrophically, heterotrophically or mixotrophically depending on the type of carbon source available in their environment (Seckbach, 2007)(Henley et al., 2007).

2.2. Major factors determining microalgae performance

2.2.1. Light availability

Light intensity is one of the main limiting factors that determine the growth of microalgae cultures. The time and intensity of light directly affect the photosynthesis of microalgae and to some extent determine the biochemical composition of microalgae and the biomass production yield (Krzemińska et al., 2014)(Acién Fernández et al., 2017). For the entire light spectrum, microalgae only use certain wavelengths of 400 to 700 nm (photosynthetically active radiation, or PAR), the photosynthetic apparatus is saturated at relatively low irradiances ranging from 100 to 200 $\mu E/m^2$-s. As exposure to solar radiation reaches values greater than indicated value, the photosynthetic apparatus may be over-saturated or photo-inhibited. A situation related to the reduction in the rate of photosynthesis that occurs at high irradiances, although the rate of photosynthesis may also decrease due to photooxidation or other causes (Carvalho et al., 2011)(Acién Fernández et al., 2017). Light intensities change inside the culture and reduce with the culture depth, thus this variation should be taken into consideration for modelling the bioreactor or system raceway. Microalgae species vary in terms of their light requirements for maximum growth and biomass accumulation (Khan et al., 2018). In modelling the outdoor or

indoor algal culture system, growth rate and biomass productivity is calculated as a function of light. (Huesemann et al., 2013).

Regardless of the design of the photobioreactor, the light incident on the surface of the photobioreactor is attenuated as the culture grows along the photobioreactor, depending on the concentration and extinction coefficient of the biomass. This attenuation of light causes different light gradients to exist, leaving the microalgae cells exposed to different light conditions depending on the light profile and mixture (Molina-Grima et al., 1999). On the other hand, tubular photobioreactors are among the most scalable and suitable for large-scale production, since they allow resolving relative volumes of the light and dark zones to change as the tube diameter increases (Molina-Grima et al., 1999).

The light intercepted by the surface of tubular photobioreactors will depend on the configuration adopted for light distribution, which considers the diameter of the tube and its spatial distribution. The variable tube diameter influences the light gradient inside the tube and, therefore, the light regime to which the microalgae cells inside the reactor are exposed. It has been reported that using small tube diameters and adequate flow velocities can improve photosynthetic performance, by permitting light integration exposure frequencies higher than 1 Hz to be reached (Brindley et al., 2016). The challenge in the design of new photobioreactors is to achieve designs that allow an optimal spatial distribution of the tubes, maximizing the amount of solar radiation intercepted, but redistributing it over a larger surface of the photobioreactor avoiding the excess of light that can induce a photoinhibition in the culture, i.e., it is necessary to cause a "light dilution", which is mainly a function of the volume of the reactor to the surface (V/S) ratio (Tredici & Zlttelli, 1998). Another aspect to consider to improve the availability and distribution of light in the photobioreactors is the optimization of the ratio between the illuminated surface/volume and the penetration length of the light (Bosma et al., 2014).

2.2.2. Temperature

In its entirety microalgae species are capable to perform photosynthesis and cell division in a wide range of temperatures, the optimum conditions being between 20ºC and 25ºC (Li, 1980). It is known that non-optimal temperatures in tubular photobioreactors cause low growth of microalgae cultures, given that the limits tolerated by most microalgae strains range from 10 to 40°C (Bitog et al., 2011)(Ras et al., 2013). Reaffirming that temperature affects photosynthesis rate and microalgae biomass productivity, it also affects pigment content (Giannelli et al., 2015), and O_2/CO_2 solubility (Raeesossadati et al., 2014).

The optimum temperature for photosynthesis of blue-green algae (cyanobacteria) is 0–20 ºC during winter and 20–30 ºC in summer. Temperature between 22 ºC and 35 ºC is beneficial for the growth of microalgae. *Chlorella vulgaris* can grow in the temperature range of 25–30 ºC and also in an extreme environment of 30–35 ºC. *Scenedesmus* species will grow in the ranges from 10 to 40 ºC. *Spirulina* species can grow in temperatures ranging from 20 to 40 ºC, but the temperature affected the protein and carbohydrate levels (Singh & Singh, 2015). *Dunaliella salina* can grow in a wide temperature range between 0 and 45 ºC (Hamed et al., 2017), its optimum growth range in laboratory cultures is between 25 and 35 ºC (Ami Ben-Amotz, 1995). *H. pluvialis* has an optimum temperature of 20˚C, above this temperature the growth rate decreases and the final maximum cell concentration at 30.5 ˚C (Giannelli et al., 2015).

Generally, closed tubular photobioreactors need cooling and heating systems to control the temperature of the culture, which are expensive systems. The heat transferred to the tubular photobioreactors is mainly through radiation: heat absorbed by the photobioreactors medium, ground radiation, air radiation, and solar (direct and diffuse) radiation (Androga et al., 2017). There is evidence of the application of several methods to regulate temperature in forobioreactors, especially in systems designed for hydrogen production research (Barbosa et al., 2001). Those methods include external cooling by water flowing and shading (Özgür et al., 2010), immersion of the reactor in a thermo-regulated water bath or basin (Carlozzi & Sacchi, 2001)(Adessi et al., 2012), and cooling of the reactors using internal coils (Boran et al., 2012)(Avcioglu et al., 2011).

2.2.3. pH and CO_2

The productivity of microalgae cultures whatever the photobioreactor used is a function of the culture conditions to which the microalgae cells are exposed. In outdoor cultivation, the location of the photobioreactor is fundamental, since it determines the average solar radiation captured and temperature available, while the concentration of dissolved oxygen and pH are a function of the design and operating conditions of the photobioreactor (Wigmosta et al., 2011)(Quinn et al., 2013)(Slegers et al., 2013). Moreover, several works propose models describing the response of photosynthetic microorganisms to solar radiation and, to a lesser extent, to other environmental factors such as temperature, pH, dissolved oxygen, etc. Photorespirometric methods can be also exploited for monitoring algae-bacteria systems and for calibrating mathematical models, allowing for a better comprehension of the interactions involved and a more accurate prediction of system efficiencies (Argun & Kargi, 2010)

Microalgae cultures show a wide range of pH tolerance depending on culture conditions. The supply of CO_2 is usually applied for the pH control of microalgae cultures. The pH has a direct effect on the physiological properties of algae and the availability of nutrients (Vincent & Silvester, 1979). The pH determines the solubility of carbon sources and minerals. In addition, it is well known that pH and dissolved oxygen influence the photosynthesis rate. Optimal pH usually ranges from 7.5 to 8.0, this parameter determines the balance between carbon species in the solution, and the mass transfer capacity and operation mode, the final inorganic carbon in the medium. It was previously demonstrated that on-demand injection of CO_2 avoids the existence of carbon limitation but inadequate pH, or large pH variations, can reduce productivity (Watanable & Hall, 1996)(Rubio Camacho et al., 1999)(García Sánchez et al., 2003).

The pH ranges are changeable, due to diurnal and nocturnal fluctuations driven by photosynthesis and respiration. During the day, photosynthesis and the use of CO_2 raise pH levels. Respiration during the dark reverses this process and lowers pH levels again (Arudchelvam & Nirmalakhandan, 2012). The pH limits that most strains can tolerate range from 4 to 9, except for cyanobacteria, which can tolerate up to pH 11 (Castenholz et al., 2001)(T. A. Costache et al., 2013)(Richmond, 2013b).

2.2.4. Nutrients

Carbon is the main nutrient required to produce microalgae. The CO_2 may be fixed from the atmosphere but this strategy limits the growth of microalgae cells, then CO_2 or industrial gases containing it must be provided. The microalgae can also grow heterotrophically using organic forms of carbon, while others possess both autotrophic and heterotrophic traits, simultaneously. Carbon can be also used in the form of soluble carbonates for cell growth, either by direct uptake or conversion of carbonate to free carbon dioxide through anhydrase carbonic activity (Cai et al., 2013).

Other relevant nutrients include nitrogen and phosphorous. Eukaryote microalgae can transform inorganic nitrogen (nitrite, nitrate, and ammonium) into organic molecules by assimilation. In this process, first, the nitrate ion turns into nitrite, and then nitrite transforms into ammonium ion as a result of reductase enzyme activities. Finally, the ammonium ion converts into the amino acid structure (Cai et al., 2013). Phosphorus has an essential role in the metabolism of algae, it is found in nucleic acids, lipids, proteins, etc. The availability of phosphorous has a direct impact on microalgae growth. In the metabolism of microalgae, phosphorus in the culture medium is preferentially incorporated in the form of H_2PO_4 and HPO_4

into organic compounds through phosphorylation, which is the generation of ATP from adenosine diphosphate (ADP), accompanied by a form of energy input (Xin et al., 2010).

Microalgae are microorganisms that can produce their energy using light and carbon dioxide through the process of photosynthesis (photoautotrophic), so they use solar energy to reduce inorganic nutrients to organic matter producing biomass (Benemann, 2013). As output, the microalgae generate biomass and oxygen in large amounts. Up to 2000 Kg CO_2, 100 Kg N, 10 Kg P and 15 Kg K are required per ton of microalgal biomass to be produced, and up to 2 tO_2 is also released during the process (F. G. A. Fernández et al., 2021).

Nutrients recovered by microalgae-based processes can partially replace the production of synthetic N and P based fertilizers. Thus, soluble forms of N and P are produced worldwide, as they are the pillars that support agricultural production. On the other hand, nitrogen is produced by utilizing atmospheric nitrogen, and microalgae-based treatment processes in large quantities are a key issue in this area (F. Gabriel Acién et al., 2016).

2.3. Photobioreactor design and modelling

The major factors influencing the biomass productivity of microalgal mass cultures are complex, thus there is not a single optimal design of photobioreactor. Indeed, the choice of a specific technology will depend on the application, location, available resources, etc. (Molina Grima, 1999)(Acién Fernández et al., 2013)(Pruvost et al., 2016)(Legrand et al., 2021). This complexity makes it necessary to develop models and simulation tools allowing to improve photobioreactors design by including the daily and seasonal variations in the culture parameters that influence productivity (Kroumov et al., 2013)(Schuurmans et al., 2015)(I. Fernández et al., 2016).

Photobioreactors are classified according to their size, the light source (solar or artificial), or shape (Pulz, 2002)(Luisa Gouveia, 2011)(Richmond, 2013b). The challenge is to optimize their design to accomplish the requirements of microalgae to be produced. Thus, open reactors have the advantage of high solar radiation availability, low power consumption and low cost. However, they have disadvantages related to easy contamination and limited control of culture conditions (temperature, pH, dissolved oxygen, nutrients, etc.). Conversely, closed reactors have the advantage of better control of culture conditions and high solar radiation availability, allowing to produce a wider portfolio of microalgae strains with less contamination risk. Though, the higher costs derived from higher power consumption and mass transfer capacity for achieving higher biomass productivity; therefore, it is possible to improve the production and quality of the resulting biomass (Norsker et al., 2011)(Acién Fernández et al., 2013).

Close tubular photobioreactors need a temperature control system to cool or heat them, which can be expensive. In addition, a fundamental aspect to improve light availability in photobioreactors is to optimize the ratio between illuminated surface/volume and light penetration length. It is to optimize the ratio between the illuminated surface/volume and the length of light penetration, i.e., depth or diameter of the tube (Richmond et al., 1993). When scaling up a reactor, the culture conditions are easily controlled on a small scale, but large-scale production can lead to commercial failures. For example, obtaining an adequate light availability and temperature is essential for the efficiency of microalgae, but it is also a function of both sufficient CO_2 supply and O_2 oxygen removal (Briassoulis et al., 2010)(Acién Fernández et al., 2013).

Many closed photobioreactor designs were developed to produce different strains. In general, such reactors can be classified into tubular, flat-plate, column and other configurations, each one with different designs and shapes (Płaczek et al., 2017). In addition, each type has advantages and drawbacks (Table 1) (Płaczek et al., 2017)(Huang et al., 2017)(Assunção & Malcata, 2020).

Table 1. Advantages and drawbacks of the tubular, flat-plate and column photo-bioreactor configurations.

advantages	drawbacks
Tubular	
Effective in the capture of solar radiation, high S/V ratio, suitability for outdoor cultivation, good productivity of biomass, the possibility of arrangement with adequate slope to harvest sunlight, and relatively low cost.	Large variations of pH, dissolved oxygen and CO_2 along the tube length (risk of photoinhibition), risk of biofilm accumulation on tube walls, high land surface requirement and high energy requirements.
Bubble column and airlift	
Precise monitoring of culture parameters, good mixing, efficient CO_2 supply and O_2 removal, high mass transfer, high efficiency of photosynthesis, cheap, compact and easy to maintain, low requisites of space, suitable for outdoor cultivation and low energy use.	The low area of light exposition that reduced then increasing the diameter, the low ratio of reactor surface to its volume, and the possibility of biofilm formation on reactor walls.
Flat-panel	
The large surface of exposition to light, high surface to volume ratio, suitable for outdoor production, high biomass productivity, ability to maintain uniform access to light across the entire volume of cultivation, relatively cheap, easy to build and a small concentration of dissolved oxygen.	An increase in production scale requires the use of numerous modules and support structures, difficulties in control of temperature, risk of fouling, the potential for the occurrence of hydrodynamic stress in some algae species and deficient scale-up.

A photobioreactor must achieve maximum productivity, for which it must provide maximum light availability, and control different parameters such as nutrient concentration, temperature, pH, dissolved oxygen, and photoinhibition. In addition, it must be easy to operate and manage, be scaled up and allow low-cost production; however, not all photobioreactors can do this efficiently. The Fibonacci type photobioreactor presents these advantages among others, favouring above all the higher light uptake (Díaz et al., 2019)(Díaz et al., 2021).

2.4. Application of microalgae in the poultry industry

Poultry production in Chile is based on meat and egg production on an industrial scale. Today it is an activity of great technological capacity and intensive production. Within the egg production process, 70% of the costs correspond to feed, being corn the main material, which conditions the price of eggs. At 20 weeks of age, pullets start laying, which lasts until month 15, when the productivity of the birds is reduced and the layers are not enough to cover feed costs (Giacomozzi, 2014).

Eggs as a result of an industrial process, and great biological value, provide high quality protein, especiallyb rich in essential amino acids. Which could promote the synthesis and maintenance of human skeletal muscle mass. This property may be of relevance for athletes and older adults, helping the latter to counteract the sarcopenia process typical of ageing. The main protein in egg white is ovalbumin, followed by ovotransferrin and others such as lysozyme (Kovacs-Nolan et al., 2005). It is also postulated that the different egg proteins could have a favourable impact on inflammation processes, as well as antimicrobial, immunoprotective, antihypertensive and antioxidant properties (Kovacs-Nolan et al., 2005)(Sanlier & Üstün, 2021). In addition, the yolk contains immunoglobulin (IgY) the main serum antibody of birds, a functional equivalent of mammalian immunoglobulin G. Both *in vitro* and *in vivo*, IgY inhibits the development of infections by gastrointestinal pathogens such as rotavirus, *Escherichia coli* and others (Kovacs-Nolan et al., 2005)(Hou et al., 2014). In addition, the lipids and phospholipids present in the yolk have been shown to have antioxidant effects and have been studied in the prevention of oxidation of unsaturated fatty acids. A phospholipid, such as phosphatidylcholine, is an important source of choline, an essential nutrient for brain development, liver function and cancer prevention. Phospholipids (PLs) with special biological activity are used to treat chronic diseases such as cardiovascular and cerebrovascular diseases (Hou et al., 2014).

The egg is also one of the main sources of vitamin D in the diet and provides numerous other nutrients such as riboflavin, folate, selenium, vitamin A and vitamin B12, among others (Gray & Griffin, 2009). These nutrients, such as zinc, selenium, retinol and tocopherols, are deficient in people who consume a mainly Western diet. Given the antioxidant capacity of these nutrients, they are potential protectors against cardiovascular disease (Cáceres & Gotteland, 2010)(Miranda et al., 2015).

Another relevant feature of the egg is its contribution of carotenoids to the human diet, such as lutein and zeaxanthin, which have antioxidant properties that protect against cataracts and macular degeneration, important causes of blindness in old age (Nimalaratne & Wu, 2015). Lutein can also influence male reproduction parameters because its addition significantly increases sperm viability (Pizzey & Bedecarrats, 2007)(Eisenhauer et al., 2017).

At present, lifestyles have been modified according to the knowledge of a healthy diet because it reduces health risks. Of this, consumers and states that have defined the issue of obesity and cardiovascular risks are associated with inadequate nutrition, as a public health problem, are aware (Mataix et al., 2003). Therefore, there has been a multiplier effect on public awareness of the preference for "healthy" functional foods (Sanz & Dalmau, 2008).

In this context, healthy egg production plays a preponderant role, as the egg yolk is important as the only carrier of fat-soluble bioactive carotenoids: lutein and zeaxanthin. The carotenoid profile of egg yolk depends markedly on the composition of the feed of laying hens (Nimalaratne & Wu, 2015). Synthetic carotenoids have been used since the 90s in European countries, to meet the requirements of consumers who prefer eggs with coloured yolks (Kotrbácek et al., 2013). As previously presented, the trend is to produce eggs with natural antioxidants, where it is possible to add in the feed a percentage of 1-2% of Chlorella microalgae in your diet, and this way the natural carotenoids are incorporated into the yolk (Kotrbácek et al., 2013).

Lutein and zeaxanthin have antioxidant and immunomodulatory functions and positively influence improving the immune system of birds (Bédécarrats & Leeson, 2006). Its supplementation in the feed of layers contributes to improving the intestinal microflora and therefore, can have a prominent role in improving the health of birds. The addition *of Chlorella vulgari*s in poultry diets has been shown to result in increased microbial diversity throughout the digestive tract, especially in the cecum (Janczyk et al., 2009)(Kang et al., 2013). Most of the studies that determine the levels of inclusion of *Chlorella* in animal feed have been associated with aquaculture, starting in this decade the feeding in birds (Ross & Dominy, 1990). In addition,

Chlorella has a growth substance known as Chlorella growth factor (CGF), which contains free amino acids, peptides, glycoproteins, polyamines, phytohormones, some vitamins, minerals and other components not yet defined that allow greater growth in birds (Doucha, 1998)(Kang et al., 2013).

Microalgae are considered efficient organisms for obtaining bioactive substances (Spolaore et al., 2006). They are organisms with a prokaryotic or eukaryotic structure, photosynthetic capacity capable of converting solar radiation into biomass with high efficiency. They have high production rates, adaptability to different conditions in which the environment is found and are ubiquitous in any aquatic environment where there is a sufficient source of carbon, nutrients and light, along with an appropriate temperature range (Wijffels & Barbosa, 2010). Microalgae are a very rich source of essential nutrients and an important source of food, especially in Asian countries, which have perfected the production of biomass whose optimal qualities have allowed feeding in animals. In its composition, microalgae stand out particularly for their high protein content, with values higher than 50% of dry weight, and an amino acid profile similar to the soybean or fish meal, being only slightly deficient in sulfur amino acids and lysine. (wiaętkiewicz et al., 2015)(Mobin & Alam, 2017)(Ramana et al., 2017).

Selected studies on the effects of *Spirulina* inclusion into poultry diets. Report Mariey et al., (2014) 1 or 3 % de of Spirulina increased body weight gain (BWG), feed efficiency, meat colour score, the weight of bursa, thymus and spleen, total protein, globulin and albumin, and red and white blood plasma cholesterol, triglycerides, and total lipid. The studio utilizes Broiler chickens, the duration of the experiment was up to 42 days, during which the authors analyze the performance, carcass and meat quality, blood haematology and biochemistry, and weight of lymphoid organs. Ragab & Fathi, (2018) determine the effects of the inclusion of *Spirulina platensis* on broiler diets on productive performance and some physiological responses. Considers commercial basal diet (control group), while the other groups were supplemented with *Spirulina platensis* (0.3, 0.5, 0.7 and 0.9 g/kg). The results obtained from the investigate showed that chickens fed 0.9 and 0.7 g/kg of *Spirulina platensis* feed had a significantly better value of body weight gain growth rate, immune organs, improvement feed conversion, blood parameters and microbial load.

Heat stress generates a negative effect on broiler productivity is mediated by the induction of oxidative stress. The microalgae Spirulina platensis has several applications in poultry nutrition, as it contains high levels of bioactive antioxidant compounds that can minimize oxidative stress damage induced by high environmental temperature Moustafa et al., (2021)

investigate the effects of dietary Spirulina inclusion at different levels on growth performance, redox status, carcass traits, meat quality, blood haematology, and metabolites profile of broilers subjected to cyclic heat stress. The best impact was observed in chickens fed 1% spirulina. Haematological results indicate an increase in haemoglobin and haematocrit levels with *Spirulina* supplementation compared to the non-supplemented stressed group. It can be concluded that the diet Spirulina supplement to 0.5 or 1% to broiler reared under heat stress conditions can effectively improve broiler production performance and balance the redox status.

Chlorella is one of the most commercially demanded microalgae, comprising 14 species (Champenois et al., 2015). This is characterized by a chemical composition that depends on the species, i.e. *Chlorella vulgaris* contains between 51-58% protein, 12-17% carbohydrates and 14-20% lipids; *Chlorella pyrenoidosa* contains 26% protein, 26% carbohydrates and 2% lipids, data expressed as % biomass dry weight (Um & Kim, 2009)(Sydney et al., 2010). In the fifties, a study of birds showed that dried *Chlorella*, included in the diet of 10% poultry, are a great source of carotenes, riboflavin and vitamin B_{12} (Combs, 1952)(Arakawa et al., 1960). *Chlorella* can improve the nutritional components of the egg and improve the colour of the yolk, as an indicator of its quality. More specifically it has been identified that the carotenoids in the microalgae are a complex mixture, the main components of which are canthaxanthin, astaxanthin and their esters, lutein and B-carotene (L. Gouveia et al., 1996). By supplementing 1% to 2% with dry *chlorella* biomass, the concentration of total carotenoids in egg yolks can be increased from 46% to 119% (Kotrbácek et al., 2013).

In a previous study Heng et al., (2021) evaluate the productive performance, egg quality, antioxidant enzyme activity, free radical scavenging capacity and gene expression of antioxidant enzymes in laying hens, by adding natural astaxanthin (ASTA) from *Haematococcus pluvialis* to the feed. The results showed that a diet rich in ASTA improves free radical scavenging capacity and antioxidant enzyme activity. In addition, it lowers plasma levels of low-density lipoprotein cholesterol and triglycerides (Gao et al., 2020). Like Spirulina, the addition of astaxanthin (*Haematococcus pluvialis*) to the broiler diet showed an improvement in the immunological characteristics of broilers reared under normal and elevated environmental temperatures (Awadh & Zangana, 2021). Astaxanthin of *H. pluvialis* did not affect the quality of fresh eggs, rather it allows to prolong the storage time of eggs (Heng et al., 2020). *Spirulina platensis* In Japanese quail, the diet had a positive effect on egg quality. This is because the diet reduced the levels of saturated fatty acids, and increased the levels of monounsaturated fatty acids, which are beneficial to the health of consumers.

Listado de publicaciones en los últimos 5 años. En caso de publicaciones con más de un autor, indicar en negrita el autor principal.	Publicación indexada ISI: Indexada 1. **Díaz, J.P**., C. Inostroza, and F.G. Acién Fernández. 2019. "Fibonacci-type tubular photobioreactor for the production of microalgae". Process Biochemistry 86 (noviembre): 1–8. https://doi.org/10.1016/j.procbio.2019.08.008 2. **Díaz, Juan Pablo**, Cristian Inostroza, and Francisco Gabriel Acién. 2020. "Scale-up of a Fibonacci-Type Photobioreactor for the Production of *Dunaliella salina*". Applied Biochemistry and Biotechnology, no September. https://doi.org/10.1007/s12010-020-03410-x. 3. **Díaz, Juan Pablo** & Yarela Flores Arévalo. Evaluating marine reserves as a management policy in the central-southern anchovy fishery (Engraulis ringens) of Chile. Latin American Journal of Aquatic Research, 49(2): 212-226, 2021. https://10.3856/vol49-issue2-fulltext-2614 4. Masatoshi Futagawa, Jessica Pizarro, German Bueno & **Juan Pablo Díaz**. Early development of the Peruvian rock seabass *Paralabrax humeralis* (Teleostei: Serranidae): morphological description of the embryonic and yolk-sac larval stages. Latin American Journal of Aquatic Research, 4 520 9 (3): 520Latin American Journal of Aquatic Research -525, 2021 https://doi.org/10.3856/vol49-issue3-fulltext-2631 5.

. Finally, antioxidants increased in egg yolks (Boiago et al., 2019)

3. MATERIAL AND METHODS

3.1. Microorganism and culture conditions

Three strains of microalgae were used: (i) *Arthrospira (Spirulina) platensis* M2 (National Research Council of Florence, Italy). (ii) *Dunaliella salina* (strain code 007, collection of the company GATTAVARA SACI, isolated from the Salar de Atacama desert), (iii) *Chlorella* sp. (UTEX # 2168, USA)(Figure *1*).

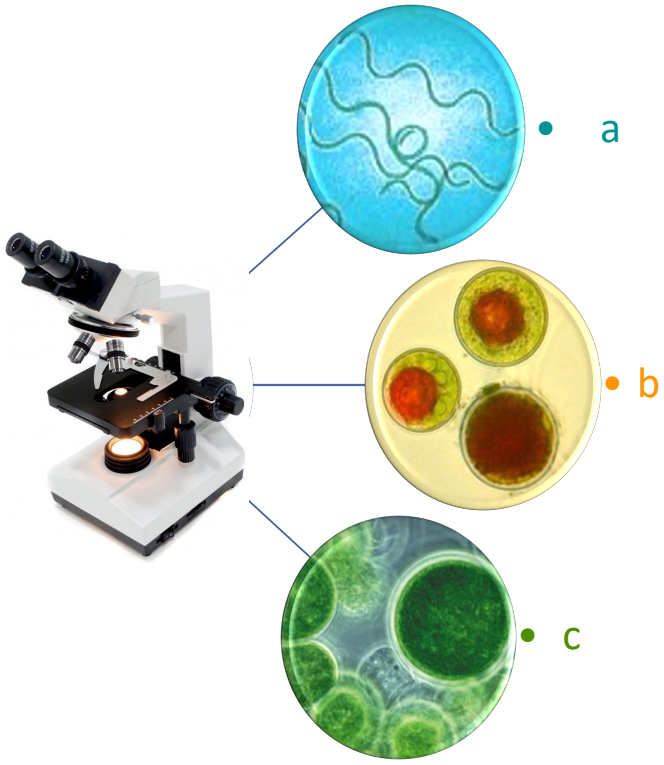


Figure 1. Microscope view of selected strains. (a) *Arthrospira (Spirulina) platensis*. (b) *Dunaliella salina* and (C) *Chlorella* sp.

The culture media used for the three strains are tested in two experimental conditions: Indoor and outdoor. For *Dunaliella salina*, four different culture media were evaluated under laboratory conditions in a Fibonacci photobioreactor all of them using KNO_3 as the nitrogen source. (**¡Error! No se encuentra el origen de la referencia.**).

Table 2. Culture media used for the three strains.

<table>
<tr><th></th><th>Indoor</th><th>Outdoor</th></tr>
<tr><td>Arthrospira platensis</td><td>Growth assays were performed using Guillard's f/2 culture medium (Aguilar et al., 2004)</td><td>A culture medium prepared using fertilizers was used ($NaHCO_3$, 8.4 g/L, sea salt 5.0 g/L, KNO_3 3.6 g/L, $(NH_4)_2HPO_4$ 0.4 g/L, $MgSO_4$ 0.02 g/L, Fe-EDTA 0.0016 g/L) (Rojas et al., 2012)*</td></tr>
<tr><td>Dunaliella salina</td><td>The culture media utilized were based on the medium already proposed (Ben-Amotz, 1995).
<table>
<tr><th>Medium</th><th>g/l</th><th>Medium</th><th>g/l</th></tr>
<tr><td>KSP</td><td></td><td>KSP Modified</td><td></td></tr>
<tr><td>KNO_3 natural origin</td><td>0.5</td><td>KNO_3 natural origin</td><td>0.3</td></tr>
<tr><td>Triple Super Phosphate</td><td>0.05</td><td>Sea salt</td><td>150</td></tr>
<tr><td>Sea salt</td><td>150</td><td>$NaHCO_3$</td><td>3</td></tr>
<tr><td></td><td></td><td>Sol. FDA (ml/l)</td><td>0.3</td></tr>
<tr><td></td><td></td><td>Sol. FDAFe (ml/l)</td><td>0.1</td></tr>
<tr><td>BRIASCU Modified</td><td></td><td>SM</td><td></td></tr>
<tr><td>KNO_3 natural origin</td><td>0.05</td><td>KNO_3 natural origin</td><td>0.06</td></tr>
<tr><td>Triple Super Phosphate</td><td>0.025</td><td>Triple Super Phosphate</td><td>0.02</td></tr>
<tr><td>Sea salt</td><td>150</td><td>Sea salt</td><td>150</td></tr>
<tr><td>$NaHCO_3$</td><td>4.2</td><td>$NaHCO_3$</td><td>0.5</td></tr>
<tr><td>FeSO4</td><td>0.025</td><td>Sol. FDAFe Jhonson (ml/l) *</td><td>10</td></tr>
<tr><td>HCL (ml/l)</td><td>1</td><td>*FeCl 0.09 µM
Na2 EDTA 0.05 µM</td><td></td></tr>
</table></td><td>The selected medium was "KSP Modified"
<table>
<tr><th>Medium</th><th>g/l</th></tr>
<tr><td>KNO_3 natural origin</td><td>0.3</td></tr>
<tr><td>Sea salt</td><td>150</td></tr>
<tr><td>$NaHCO_3$</td><td>3</td></tr>
<tr><td>Sol. FDA (ml/l)</td><td>0.3</td></tr>
<tr><td>Sol. FDAFe (ml/l)</td><td>0.1</td></tr>
</table></td></tr>
<tr><td>Chlorella sp.</td><td>The Chlorella was maintained in the laboratory using BG-11 culture medium (Bolld, 1969)</td><td>The production was based on three agricultural fertilizers: $Ca(H_2PO_4)_2$ 1.2 g/L, NO_3K 0.80 g/L and agricultural micro-elements 0.05 g/L</td></tr>
</table>

* This culture medium was checked to provide an excess of nutrients.

The indoor and outdoor experiments were developed at the Universidad Arturo Prat and collaborating companies in the Salar Atacama (Table 3).

Table 3. The indoor and outdoor experiments.

Institutions	**Universidad Arturo Prat**	**GATAVARA SACI Tirana**	**CORPESCA S.A Arica**
Microalgae	*I. [1,2]Arthrospira (Spirulina) platensis* *II. [1]Dunaliella salina* *III. [1]Chlorella sp*	*Dunaliella salina*	*Chlorella sp*
Condition	[1]Indoor and [2]Outdoor	Outdoor	Outdoor
Georeferenced	Log. 70 07' 52 " O Lat. 20 16' 13 " S	Log. 68 16' 13" O Lat. 22 53' 51" S	Log. 70 19' 08" O Lat. 18 30' 16" S
Photography			

.

3.2. Design and scale-up of the photobioreactor and operation conditions

The design of the Fibonacci-type photobioreactor was developed based on the biometric parameters of the shells of marine organisms, which follow the Fibonacci geometry pattern. Employing the maximum light exposure criterion (Calabrò, 2013), a three-dimensional structure of the shell was modelled, and then improved by adjusting the global geometry to the site where the installation was planned in Tarapacá, Chile. Subsequently, the design was completed with the design of the support structures for the interior and exterior units and then developed their design and construction drawings (Figure 2).

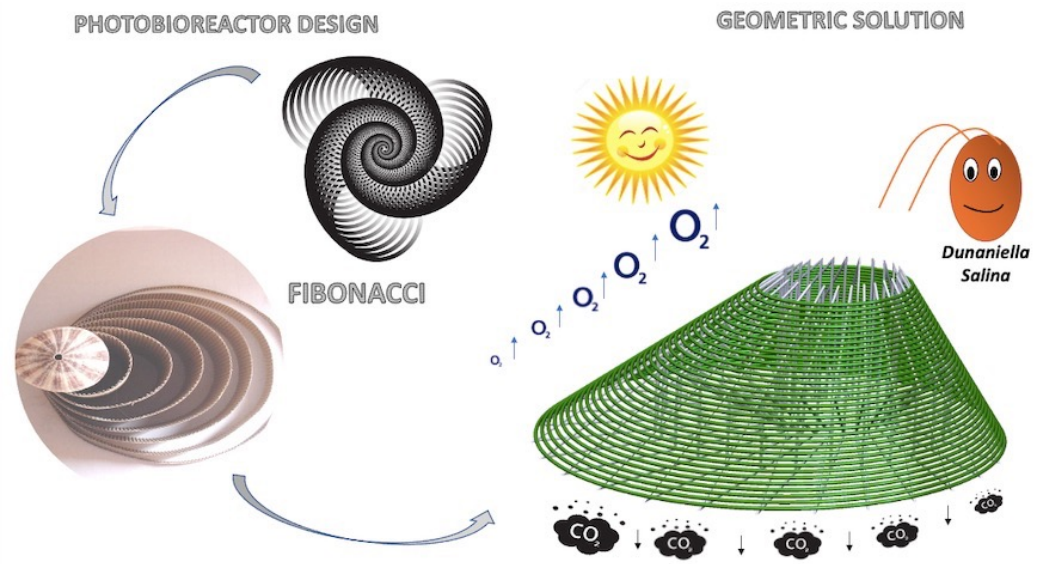


Figure 2. The design of the Fibonacci-type photobioreactor geometric solution (Biometric of marine organisms and three-dimensional structure).

For *Arthrospira (Spirulina) platensis* and *Dunaliella salina*, their growth was first evaluated under laboratory conditions. The test was carried out in a Fibonacci-type photobioreactor with a capacity of 10 L (Figure 3), the design parameters are presented in Table 4.

Figure 3. Fibonacci-type photobioreactor with a capacity of 10 l.

Table 4. Design parameters in Fibonacci-type photobioreactors at different capacity volumes.

Parameters	Laboratory 10 L (*Spirulina*)	Outdoor 290 L (*Spirulina*)	Outdoor 1250 L (*Dunaliella*)	Outdoor 2500 L (*Chlorella*)	Units
Tube diameter	0.0126	0.0252	0.0378	0.052	m
Tube length	47	501	844	910	m
Land length (L)	0.96	3.6	7.5	10	m
Land width (w)	0.8	2.8	5.8	7.7	m
Degassing tank	0.002	0.04	0.3	0.57	m^3
Surface tube exposed to light (St)	1.9	39.7	100.2	148.7	m^2
Land occupied (S)	0.77	10.08	43.50	77.00	m^2
Volume tube (Vt)	0.01	0.25	0.95	1.93	m^3
Volume reactor (V)	0.01	0.29	1.25	2.50	m^3
V/S	10.23	28.76	28.67	32.50	L/m^2
Vt/St	3.15	6.30	9.45	13.00	L/m^2
%Vdark	0.25	0.14	0.24	0.23	
St/V	236.7	136.8	80.4	59.4	m^2/m^3

The Fibonacci-type photobioreactor has been scaled up in three locations: Iquique, Arica and la Tirana, all of them close to the Atacama Desert. Thus, the photobioreactor r was scaled up in very diverse environmental conditions and with different strains (**¡Error! No se encuentra el origen de la referencia.**). The criteria for the scale-up process was to maintain the ratio of diameters of the upper and lower ellipses at 1.3 (Figure *4*), to maintain the proportions by increasing sizes and improving the Vt/St ratio. In the scaling up of the Fibonacci type photobioreactor, the tube diameter increased and also the surface occupied by the photobioreactor. The irradiance, temperature, pH and dissolved oxygen in the culture were evaluated at each step of scale-up, also evaluating the yield in terms of biomass concentration and productivity.

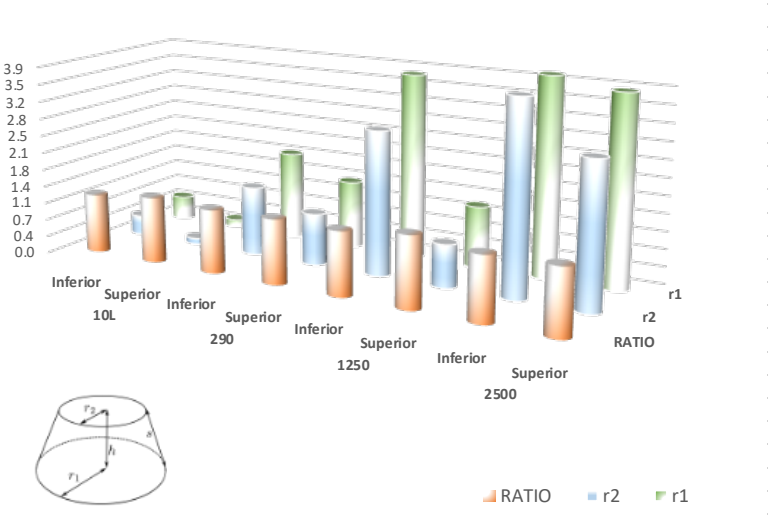
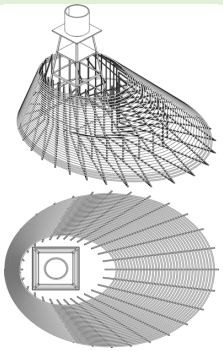
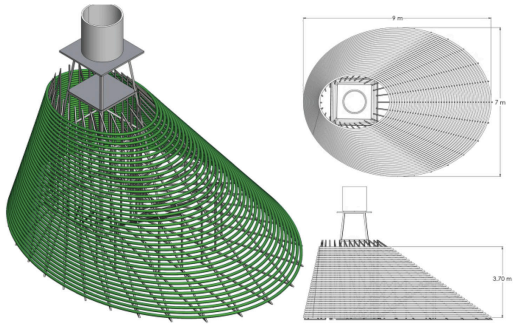
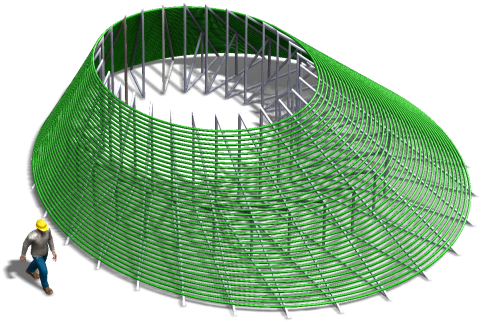
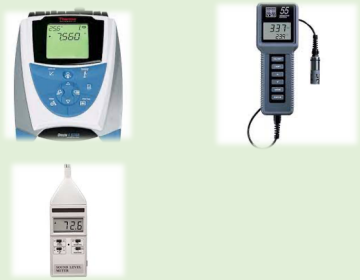


Figure 4. Ratio between the radio of the upper and lower ellipses, with an average of 1.3 in the photobioreactor scaling.

Table 5. Characterization of the photobioreactors and operating conditions (T, pH, CO_2 and O_2).

Institutions	**Universidad Arturo Prat**	**GATAVARA SACI Tirana**	**CORPESCA S.A Arica**
Strain	*Arthrospira (Spirulina*	*Dunaliella salina*	*Chlorella sp*
Condition	Outdoor	Outdoor	Outdoor
Capacity	290 L	1250 L	2500 L
Photobioreactor modelling			
Photography			

Institutions	**Universidad Arturo Prat**	**GATAVARA SACI Tirana**	**CORPESCA S.A Arica**
Operating conditions (T, pH, CO_2 and O_2)	The reactor was naturally illuminated with the solar irradiance being determined by the light availability present at the north-facing installations of the Universidad Arturo Prat (Iquique, Chile) where the reactor was located. The pH was also controlled by injecting CO2 on demand, while dissolved oxygen and temperature were not controlled. Systematically the daily variation of irradiance and culture parameters were recorded. To accomplish this, pH (111500 4-STAR, Thermo Orion, UK), temperature and dissolved oxygen (55,YSI, USA) sensors were positioned at the end of the solar collector where extreme values are registered. In addition, environmental conditions such as air temperature, humidity and solar radiation were measured (Radiometer 840029, Sper Scientific, USA).	The Fibonacci-type photobioreactor has been scaled up at a site in the Atacama Desert where temperatures fluctuate between − 5 and 40 °C, with an average of 330 clear days per year. The average maximum solar radiation is 1752 μEm^{-2} s^{-1} (Ministerio de Energía, 2019). The CO_2 is injected at the base of the truncated half-cone. At the end of the ascending spiral is the impulsion system, which in this case is "air-lift" with air supplied by a "Blower" S-41 (Sweetwater Inc., USA). The photobioreactor spiral was distributed in three tube segments to reduce oxygen accumulation. 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, CO_2 and air flow rates provided were 0.001 and 0.01 v/v/min, respectively. Physical and chemical variables were recorded at the end of the spiral leading to the gas exchange pond. The variables recorded were pH (1115001 4-STAR, Thermo Orion, UK), temperature, dissolved oxygen and the oxygen saturation percentage (55,	Temperatures at this location range from 25.4 to 19.3 °C in summer and from 18.7 to 14.5 °C in winter. The cloud frequency is 13% and the maximal solar radiation at noon ranges from 500 to 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The angle of exposure of the spiral tube surface was changed, correcting it towards the latitude and longitude of the location to maximise the capture of solar radiation (Jacobson & Jadhav, 2018). The photobioreactor consists of three major parts, i.e. photoactive panel, drive system and gas exchange pond. The air-lift drive system was replaced by a Double Diaphragm Pump (MK25, Jofee Dump Co. LTD, China). To monitor the culture conditions inside the reactor different sensors were allocated at the inlet and outlet of the spiral tube (solar collector). A pH sensor (WQ-201, Global Water Instrumentation, USA) was used to measure the pH. To monitor temperature and dissolved oxygen an optical sensor (WQ FDO 925, Global Water Instrumentation, USA) was used. Solar radiation was measured using a radiometer (Radiometer 840029, Sper Scientific, USA). All sensors were integrated using a Supervisory Control and Data Acquisition (SCADA).

Institutions	**Universidad Arturo Prat**	**GATAVARA SACI Tirana**	**CORPESCA S.A Arica**
		YSI, USA) and Radiometer 840029 (Sper Scientific, USA	
***pH, O_2,* Radiometer**			
Impeller system	air-lift system	air-lift system	Double Diaphragm Pump
			
Data logger	At 09:00 h, 13:00 h and 18:00 h.	At 09:00 h, 13:00 h and 18:00 h.	Automatic
The photoactive panel for reactors	In this case, it corresponds to a single polyvinyl chloride tube, transparent and manufactured with characteristics of resistance to ultraviolet radiation; also, pH levels from 3 to 14 and temperatures from 0 to 45°C° can tolerate strongly saline water.		

3.3. Laboratory measurements

Table 6 summarizes the analytical methods and equipment utilized during the experiments.

Table 6. Analytical methods and equipment utilized.

Selected strains	Analytical Methods	Instrument
Arthrospira (Spirulina) platensis	Dry biomass was measured daily, for that 20 mL of culture sample was filtered, washed with HCl at pH=5 and then dried in an oven at 60 ºC for 12 h (in triplicate). Additionally, 10 mL of culture sample was taken to determine the absorbance of the cultures (Spectrophotometer SP 830 Plus, Metertech, Taiwan). Moreover, a daily observation was performed by microscope to determine the cell chain characteristics such as the number of turns, the filament shape, the chain length and the overall aspect of the cells.	
Dunaliella salina	The biological variables of the cultures were recorded daily. The appearance of the cells was observed with an optical microscope. The dry weight content was determined by sampling 30 mL of culture and filtering it through a pre-combusted (550 ºC, 2 h) and pre-weighed glass fibre filter (0.45 µm, Whatman), and then it was washed with ammonium 0.5 M, dried at 105 ºC and it is left to cool for a while in a desiccator and then weighed. The absorbance was measured with a spectrophotometer (SP 830 Plus, Metertech, Taiwan) at 750 nm. The cell density (cells/mL) was recorded daily for each sample; the cells were counted in a Neubauer chamber (0.1 mm depth).	 
Chlorella sp	The biomass dry weight was daily measured by taking 100 ml samples of the triplicate culture from the gas exchange tank. The samples were filtered with preweighed filter paper (GF/C, 47 mm, Whatman). The filtered samples were rinsed with distilled water and dried at 60 °C for 24 hours, allowed to cool in a desiccant and then reweighed. In addition, three 10 ml samples were used to measure absorbance at 680 nm with a spectrophotometer (SP 830 Plus, Metertech, Taiwan), to correlate the dry weight and absorbance measurements. Cell density (cells/ml) was counted for each sample in a Neubauer chamber (0.1 mm deep).	  

3.4. Light availability and photosynthetic efficiency

The average irradiance to which the microalgae cells are exposed inside the photobioreactor was determined. This irradiance is calculated from the total radiation intercepted by the surface of the photobioreactor (Io), the radius of the tube (R), and the light attenuation by the cells which is a function of light attenuation coefficient (Ka) and biomass concentration (Cb). To calculate the average irradiance the simplified Equation 1 was used (Grima et al., 1994).

$$Iav = \frac{Io}{Ka \cdot Cb \cdot R}\left(1 - exp(-Ka \cdot Cb \cdot R)\right) \qquad \textit{Equation 1}$$

To estimate the specific growth rate (μ) the Equation 2 (Grima et al., 1994) is utilized, it is a function of the maximum growth rate (μmax), the light availability (I) on the photobioreactor, and the irradiance constant of the microorganism (Ik).

$$\mu = \frac{\mu max * I^n}{Ik^n + I^n} \qquad \textit{Equation 2}$$

To evaluate whatever reactor it is fundamental to estimate the quantum yield and photosynthetic efficiency, so analyzing the light utilization efficiency in the photobioreactor (F. Gabriel Acién et al., 2016)(Romero Villegas et al., 2017). Quantum yield (ψ) is defined as the amount of biomass generated by a unit of solar radiation absorbed usually expressed in photon molar or Einstein (Molina Grima et al., 1997). It is calculated as the relationship between biomass volumetric productivity (Pb) and the photon flux absorbed in the unit volume (Fvol) using Equation 3. The absorbed photons (Fvol), can be estimated as a function of the average irradiance (Iav), the biomass concentration (Cb) and the light attenuation coefficient (Ka) (Equation 4). This coefficient in energy units (ψE) can be converted into photosynthetic efficiency units (ψKJ), considering the average energy of the light used ($KJ \cdot E^{-1}$) Equation 5. The bioenergetics yield (ψE) represents the percentage of light energy that is converted to chemical energy, and it can be calculated as the product of photosynthetic efficiency (ψKJ) by the combustion heat of biomass (Qb) (20 MJ/kg).

$$\psi_E = \frac{Pb}{Fvol} \qquad \textit{Equation 3}$$

$$Fvol = I_{av} * C_b * K_a \qquad \textit{Equation 4}$$

$$\Psi_{KJ} = \Psi_E Q_b$$ *Equation 5*

3.5. Simulation of the performance of Fibonacci-type photobioreactor

To simulate the performance of the Fibonacci type photobioreactor in different scenarios (tube length, tube diameter) the oxygen production rate and dissolved oxygen accumulation in the cultures were calculated. For that, the hyperbolic model was utilized. This model allows to estimates the oxygen production rate by photosynthesis (PO_2) (**¡Error! No se encuentra el origen de la referencia.**) (Brindley et al., 2016; T. A. A. Costache et al., 2013) as a function of maximal oxygen production rate (PO_{2max} = 4.6·10-5 molO_2/g biomass min, equivalent to 2.73·10^{-3} mgO_2/L s), an exponential factor (n=2) and irradiance constant (Ki= 70 µE/m^2s). The maximal length of the reactor is determined by the maximal dissolved oxygen concentration tolerable for the selected strain, it is calculated using **¡Error! No se encuentra el origen de la referencia.**. This equation considers the increase of dissolved oxygen concentration taking place in each increment of the length (L_i - L_{i-1}) due to photosynthesis, the final dissolved oxygen concentration achieved is also a function of liquid velocity (V_Q). The objective is to define the length maximum of the tube allowing to ensure that dissolved oxygen concentration does not exceed 200 % oxygen saturation. Simulations were performed at different biomass concentrations and tube diameters, also different volumes of the reactor. For each simulation, the final volumetric biomass productivity (Pb) is calculated (Equation 8).

$$\mathrm{PO}_2 = \frac{PO_{2\,max} * Iav^n}{Ki^n + Iav^n}$$ *Equation 6*

$$\mathrm{DO}_2 = O_{2\,Max\,sat} + \sum_{i=1}^{imax} \frac{PO_2 * (L_i - L_{i-1})}{V_Q}$$ *Equation 7*

$$\mathrm{P}_b = Cb * \mu$$ *Equation 8*

3.6. Software

Data were processed with Microsoft Excell 2016. The SolidWorks module used was Flow Simulation activating the model number of Solar Radiation, the dominant equations of the iteration are: Solar direction vectors are calculated using the following Equation 9. $\vec{Z}$ is the zenith direction, $\vec{N}$ is the north direction, $\vec{E} = \vec{N} \cdot \vec{Z}$ is the east direction; θ_s is the solar elevation angle. That is the angle between the direction of the geometric centre of the sun apparent disk and the (idealized) horizon; $\emptyset_s$ is the solar azimuth angle. That is the angle from due north in a clockwise direction. The solar elevation θ_s can be calculated, to a good approximation, using

Equation 10. Where φ is the local latitude, δ is the current sun declination, $h = \frac{t}{86400} 2\pi - \pi$ is the hour angle of the present time t[s] (for example, at solar noon 12:00 am, t = 43200 s and hour = 0). The solar azimuth angle $\emptyset_s$ can be calculated, to a good approximation, using the following Equation 11 and Equation 12.

$$\vec{S} = \left(\vec{E} \cdot sin\, \emptyset s + \vec{E} \cdot cos\, \emptyset s\right) cos\, \emptyset s + \vec{Z} sin\, \emptyset s \qquad \textit{Equation 9}$$

$$sin\, \theta_s = cos\, h\, cos\, \delta\, cos\, \phi + sin\, \delta\, sin\, \phi \qquad \textit{Equation 10}$$

$$sin\, \emptyset\_s = -\frac{sin\, h\, cos\, \delta}{cos\, \emptyset_s} \qquad \textit{Equation 11}$$

$$\sin \emptyset_s = \frac{\sin \delta \cos \phi - \cos h \cos \delta \sin \phi}{cos\, \theta_s} \qquad \textit{Equation 12}$$

The intensity of solar radiation is calculated by Equation *13*, where Cm = 0.092 and $m_i = \frac{1.029}{\sin \theta_s + 0.029}$ are empirical values; n is the cloudiness i.e. the presence of clouds in the sky and ranges from 0 (n clouds in the sky) to 1 (all the sky is covered by clouds); Qso=1360 W/m^2 is the solar constant (i.e. the energy reaching the Earth from the Sun at the top of the atmosphere).

$$Q_s \theta_s = \frac{Q_{so}}{1 + C_m m_i} * (1 - n) \qquad \text{Equation 13}$$

3.7. Evaluation of microalgae as a supplement in poultry feed

The experiment was carried out at the Canchones campus of the Arturo Prat University, in the Atacama Desert Chile, it began with 150 Hy line Brown hens from 22 weeks old to 36 weeks. The birds were distributed in five cage-free pens. Ambient temperature ranged from 8± 0.1 y 32 ± 0.1 °C, in the Atacama Desert lat. 20° 16´ 19,6" S AND long. 70° 07´ 34,0" W y the light cycle was 16 hours of light and eight hours of darkness. The hens were randomly distributed in the five chicken coops with five dietary treatments and five replicas of each (

Table 7). Each of the chicken coops measured 6 L × 3 W × 2 m H with an indoor stocking density of 0.72 hens/m^2. The hens were fed diets in which microalgae Spirulina is incorporated into the feed at 1% of the daily feed and 20, 40 and 60 ppm of the natural astaxanthin (ASTA) from Haematococcus pluvialis depending on the treatment (T1). Spirulina microalgae were grown in a raceway system in collaboration with the company SOLARIUM BIOTECHNOLOGY S.A. and natural astaxanthin was supplied by ATACAMA BIO NATURAL PRODUCT S.A.

Table 7. Experiment design

	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)	Treatment 4 (T4)	Treatment 5 (T5)
Use of microalgae	**Control Group: no microalgae**	**1% spirulina and 20 ppm ATS (kg/Feed)**	**1% spirulina and 40 ppm ATS (kg/Feed)**	**1% spirulina and 60 ppm ATS (kg/Feed)**	**1% spirulina (kg/Feed)**
	T1	T 2	T3	T 4	T5
Repetición	5	5	5	5	5
Repetition	5	5	5	5	5
Repetition	5	5	5	5	5
Repetition	5	5	5	5	5
Repetition	5	5	5	5	5
Total birds in test	150				

The basic diet is composed of corn, dehulled soybean meal, and corn gluten meal. The diet was formulated based on the recommendations of the management manual for layers Hy-Line Brown. Corresponding to the period of 22 to 36 weeks (Table 8). The water was provided *ad-libitum.*

Table 8. Ingredient composition of experimental diets

Ingredients	Content % (22 to 36 wk)
Corn	64
Soybean meal	20
Corn gluten meal	0.38
DL-Methionine	0.35
Lysine HCL	0.24
Fat animal vegetable blend	2.75
Limestone	9.76
Mono-Dical PO4	1.66
Salt	0.29
Sodium bicarbonate	0.12
Vitamins[1]	0.27
Calculated nutrient composition (%)	Content % (22 to 36 wk)
E. Metaboliz (Kcal/kg)	2911
Crude proteín	15
Calcium	0.94
Phosphorous	0.43
Sodium	0.17

[1]Vitamin premix added at this rate yields (per kg): vitamin A, 8.8 IU; vitamin D3, 3.3 IU; vitamin E, 16 IU; vitamin K3, 2.2 mg; vitamin B12, 0.022 mg; vitamin K3, 2.2 mg; thiamine (B_1), 1.7 mg; riboflavin (B_2), 5.5 mg; niacin (B_3), 28 mg; pantothenic acid (B_5), 6.6 mg; pyridoxine (B_6), 3.3 mg; Mn, 88 mg; Zn, 88 mg; I, 1.7 mg; Cu, 5.5 mg; Fe, 55 mg.

To determine the live body weight gain of birds LBWG (Kg/bird) in the study period. The following formula was used $LBWG_{22\text{-}36} = LBWG_{36} – LBWG_{22}$. The growth rate (GR) was calculated as follows: $GR_{22\text{-}36}$= (LBW36–LBW22)/0.5(LBW22+LBW36). The pullets were randomly marked for identification, and their data record was observed from 22 and 36 weeks.

The quality of eggs produced when feeding poultry with feed containing microalgae was evaluated in the Avian Pathology Laboratory of the University of Chile. Egg quality was measured through the techniques of:

- Measurement Of eggshell resistance: Shell thickness in three zones and eggshell weight, performed on the machine EggForce®.

- Physical Determinations: The weight of the egg, and the colour of the yolk by DSM Yolk Color Fan (formerly Roche Yolk Color Fan), is 16 scales colour index to distinguish the yolk colour density and is widely used in the poultry industry all over the world. It is analyzed through the EggAnalizer® machine.

The pure liquid yolk was mixed with distilled water in a ratio of 1:1.5 (v/v) and the solution was adjusted to pH 7.0 with NaOH 0.1 M and centrifuged at 12000 rpm for 45 minutes at 4° C. Obtaining granules and plasma that were freeze-dried separately. For carotenoid extraction was used with commercial sunflower oil (Carpintero-Pérez, 2021). Carotenoids were quantified using a UV-Visible spectrophotometer (Hanna Iris-HI801, United States), at the wavelength of 450 nm (Islam & Schweigert, 2015). From the absorbance values obtained in the spectrophotometric measurement, a pattern line of ß-Carotenes (Sigma Aldrich, 22040-1G-F) in sunflower oil (Carpintero-Pérez, 2021). For extraction, it is made at a temperature of 55 °C in a water bath and centrifugation at 12500 rpm for 15 minutes, obtaining granules and plasma. A total of 27 g of sunflower oil and poured into the glass at a temperature of 55 °C. Once the oil reached the desired temperature, 3 g of the sample (granules or plasma) was weighed and added to the glass of the oil. These samples were centrifuged at 13000 rpm for 7 minutes and the supernatant was collected, in which the carotenoids are found; which were quantified by measuring the absorbance of the supernatants at 450 nm, using girasol oil as a target.

To determine the concentration of total carotenoids extracted from the absorbance values obtained, a pattern line was performed using β-carotene (Sigma Aldrich, 22040-1G-F) at concentrations up to 33 µg/mL, whose absorbance was measured at 450 nm as well as the extracted samples. Substituting the absorbance value in the equation obtained from the linear adjustment and taking into account the dilutions performed, the µg of carotenoids per gram of sample (plasma or granules) were calculated.

Los datos se expresaron como la mean ± SEM. The layers were individually treated as an experimental unit, in order to determine the parameters, and all data were analyzed by a one-way ANOVA using SPSS 18.0 (IBM Corp., Armonk, NY). Statistical significance was defined at $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1. Scale-up of Fibonacci-type photobioreactor and light capture and distribution

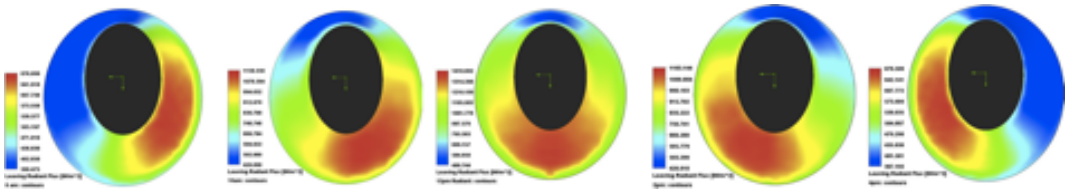
When comparing photobioreactor performance, the most important variables to consider are: available irradiance, illuminated surface area and photobioreactor volume (Tredici & Zlttelli, 1998). In higher plants, the form and position of leaves are very important, and it is optimized to achieve the best photosynthetic performance. It is known that under outdoor conditions, the photosynthetic process is subject to strong light saturation effects, and the excess light inhibits the microalgal growth (Baroli & Melis, 1996) (Richmond, 2004b)(Torzillo et al., 2015). Consequently, nature has evolved helical growth strategies to dilute the flow of photons on the plant's photosynthetic surface (Falstesr & Westoby, 2003)(Fields et al., 2006). The major factor determining the performance of any microalgae culture is light availability, a function of (i) the reactor location - determining the total radiation on the horizontal surface, and (ii) the reactor design - determining how much irradiance is collected concerning that received on the horizontal surface. In raceway reactors, the total radiation available is limited to that received on the horizontal surface, but in tubular photobioreactors, more irradiance can be intercepted.

The success of scale-up processes is largely a utility of the adequate selection of parameters to be used. The objective of scale-up must be to achieve a larger size moreover as providing the same culture conditions that at a small scale. Guidelines for optimal tubular photobioreactor design include (i) uniform culture illumination (Scoma et al., 2012), (ii) low dark to illuminated volume ratio (Perner-Nochta & Posten, 2007), (iii) low O_2 partial pressure (Perner-Nochta & Posten, 2007)(Oncel & Sabankay, 2012), (iv) high illuminated surface-to-volume ratio (St/V) (Richmond, 2013a), (v) high ratio between the illuminated area and the ground area occupied by the reactor (St/S)(B. Wang et al., 2012)(Daliry et al., 2017), (vi) reduced mixing time (Oncel & Sabankay, 2012) and (vii) turbulent flow.

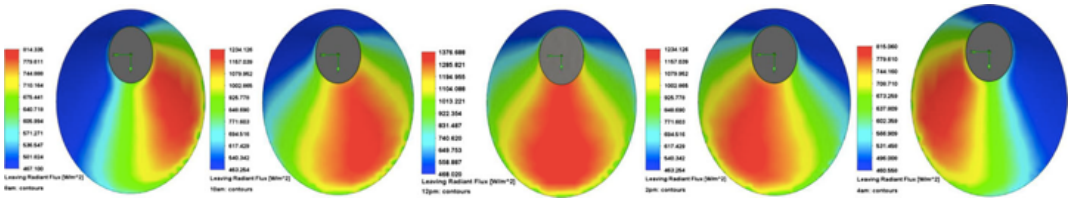
Making the photobioreactor design closer to the natural architecture of trees would offer improved light exposure strategies for diluting the flow of photons on the photosynthetic surface of the photobioreactor (Scoma et al., 2012). Thus, the Fibonacci-type photobioreactor is inspired by the frond spiralling growth strategy of the trees, 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 larger irradiances inhibition can take place.

The solar irradiance intercepted by the surface of the Fibonacci-type reactor varies throughout the day depending on the position of the sun. Maximal irradiance is intercepted by the reactor surface when it is oriented towards the sun; in contrast, the surface behind the large surface exposed to the sun receives low irradiation. The maximal intercepted irradiance by photobioreactors of 290 L, 1250 L and 2500 L is up to 1.3, 1.7 and 1.63 respectively higher than that received on the horizontal surface. The larger solar radiation interception of the photobioreactor of 1250 L is due to the increased angles that modify the reactor surface. It is also largely a function of orientation and geographical location (Sierra et al., 2008)(Figure *5*).

290 L (Vt/St = 6.3 L/m2)



1.250 L (Vt/St = 9.45 L/m2)



2500 L (Vt/St = 13 L/m2)

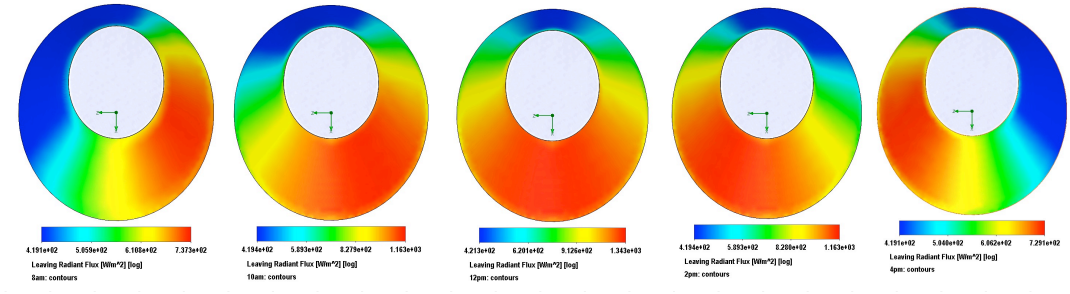


Figure 5. Solar radiation intercepted by the photobioreactor surface at 290L, 1250L and 2500L scales at different times of the day. (8:00, 10:00, 12:00, 14:00 and 16:00). Performed using SolidWorks Flow Simulation (Geographic location Iquique, Tirana and Arica, Atacama Desert).

These figures are much higher than those previously reported for the flat-panel reactor, on which intercepted irradiance is 1.22 times higher than a horizontal surface. The vertical north/south-oriented flat-panel reactors exhibited the highest solar energy interception when compared to east/west-oriented flat-panel and horizontal reactors. Although the results vary depending on the location, at latitudes below 30º, the vertically arranged flat-panel capture up to 10% more light than the horizontal surface, all year round (Sierra et al., 2008). The increased solar radiation intercepted by flat panels is also a function of the distance between them- for panels up to 1.7m in height, the optimal distance is 1.5m (San Pedro et al., 2016)(Scoma et al., 2012). In vertical tubular photobioreactors, the increase in intercepted irradiation is only 10%, but considering an optimal distance between the solar loops of the vertical tubes of 1.7 m in height 1.5m (San Pedro et al., 2014).

The Fibonacci-type photobioreactors of 290 L, 1250 L and 2500 L here presented have Vt/St ratios of 6.3, 9.45 and 13 L m^{-2} respectively, much lower than values already reported for other closed photobioreactor designs as flat-panel and tubular, which range from 50 to 150 L m^{-2} (Acién Fernández et al., 2013). Concerning open reactors, this V/S ratio is also much lower than the regular value of 200 L m^{-2} found in raceway reactors when operating at 0.2 m water depth, but is proportionally similar reported for thin-layer reactors then operating at 0.02 m water depth (20 L m^{-2}), although, on this thin-layer reactor, V/S ratio up to 5 L m^{-2} has been reported when operating at 0.005-m water depth (Masojídek et al., 2015). It is important to note that in general, a higher biomass productivity per land surface occupied is reported when the V/S ratio reduces (Masojídek et al., 2015).

Extending this analysis to annual values, the results confirmation that the overall solar radiation received on the three reactors' surfaces is more homogeneous in summer and spring; conversely, in winter and autumn, greater light gradients on the reactor surface occur (**¡Error! No se encuentra el origen de la referencia.**). The greater homogeneity in summer and spring favours optimal light utilization under these conditions when excess light can even induce photo-inhibition. Concerning the total intercepted solar radiation values, the results show that the proposed Fibonacci-type photobioreactor intercepts more light than the horizontal surface on an annual basis, especially in the summertime while distributing it in a more homogeneous form, as previously shown (**¡Error! No se encuentra el origen de la referencia.**).

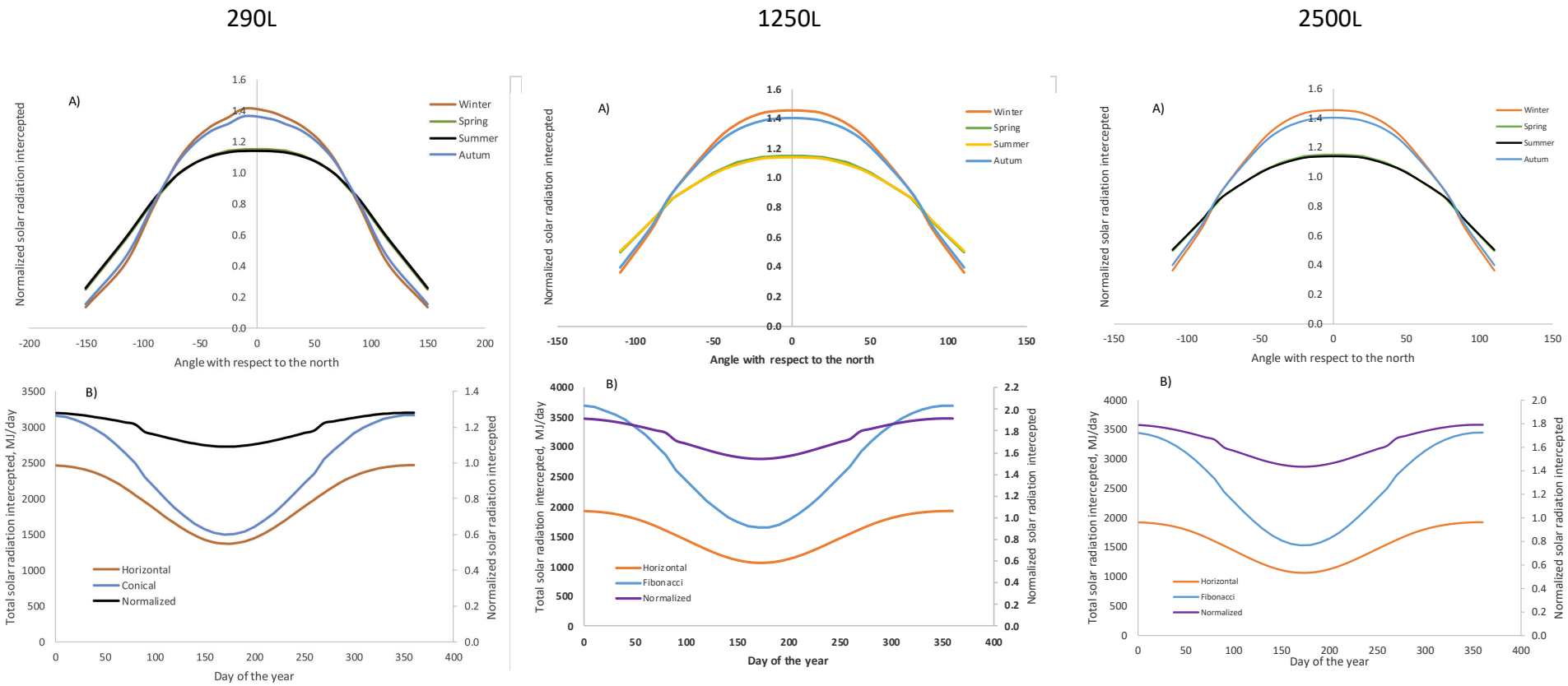


Figure 6. A) Normalized irradiance on the surface of the conical reactor concerning that received on the horizontal surface as a function of the surface angle in different seasons; B) Total solar radiation intercepted by the conical reactor and that received on the horizontal surface throughout the year and the normalized value concerning the horizontal.

4.2. Performance of the Fibonacci-type photobioreactor under indoor conditions

To analyse the performance of *Spirulina platensis* and *Dunaliella salina* in the Fibonacci-type photobioreactor, an experiment was performed under laboratory conditions using the small-scale reactor. Culture conditions of these experiments are summarized in Table 9.

Table 9. Operation conditions during the experiments performed.

	Arthrospira (Spirulina)	*Dunaliella salina*
Irradiance and laboratory temperature	The reactor was artificially illuminated at 175 µE $m^{-2}s^{-1}$ (photoperiod 16L:8D) using fluorescent lamps, a room temperature of 32 °C, and the culture temperature was 32± 1 °C (Figure 7).	The irradiance on the reactor surface was 106 µE m^{-2} s^{-1}, whereas the temperature in the laboratory was kept at 32 °C and the culture was 32 ±1 °C (Figure 7).
pH, CO_2 and %Sat. O_2	The pH was maintained at 9.1±0.2 by on-demand CO_2 injection, and the maximal daily dissolved oxygen concentration was 130 %Sat (Figure 7). In the proposed design, oxygen degassing was enough to keep the maximum daily dissolved oxygen concentration below 150%Sat., thanks to the upward flow and aeration in the tank reservoir.	The pH was maintained at 8.0±0.1 by the on-demand injection of CO_2. No dissolved oxygen was accumulated inside the reactor; values below 120%Sat. were measured. Based on oxygen accumulation into the loop, an oxygen production rate up to 6.7 mgO_2 L^{-1} h was estimated (Figure 7).
Biomass concentration and productivity	A maximal biomass concentration of 1.85 g/L was achieved at the end of the batch culture (10 days), whereas maximal daily biomass productivity of 0.30 g/L day was obtained at the linear growth phase (6 days) (**¡Error! No se encuentra el origen de la referencia.**). This biomass productivity was limited by the low irradiance (175 µE/m^2 s) on the reactor surface (artificial light provided by florescent tubes) (Figure 7).	A maximal biomass concentration of 0.96 g/L was measured at the end of the batch culture, while the maximum biomass productivity achieved was 0.12 g/L·day and 2.41 g/m^2 day on day 4 (Figure 7). The biomass concentration is double that previously found in a raceway reactor (0.3 g/L) belonging to the Norbiotech company. However, biomass productivity in the laboratory-scale reactor was limited by the low irradiance captured at the reactor surface (175 µE/m^2 s).

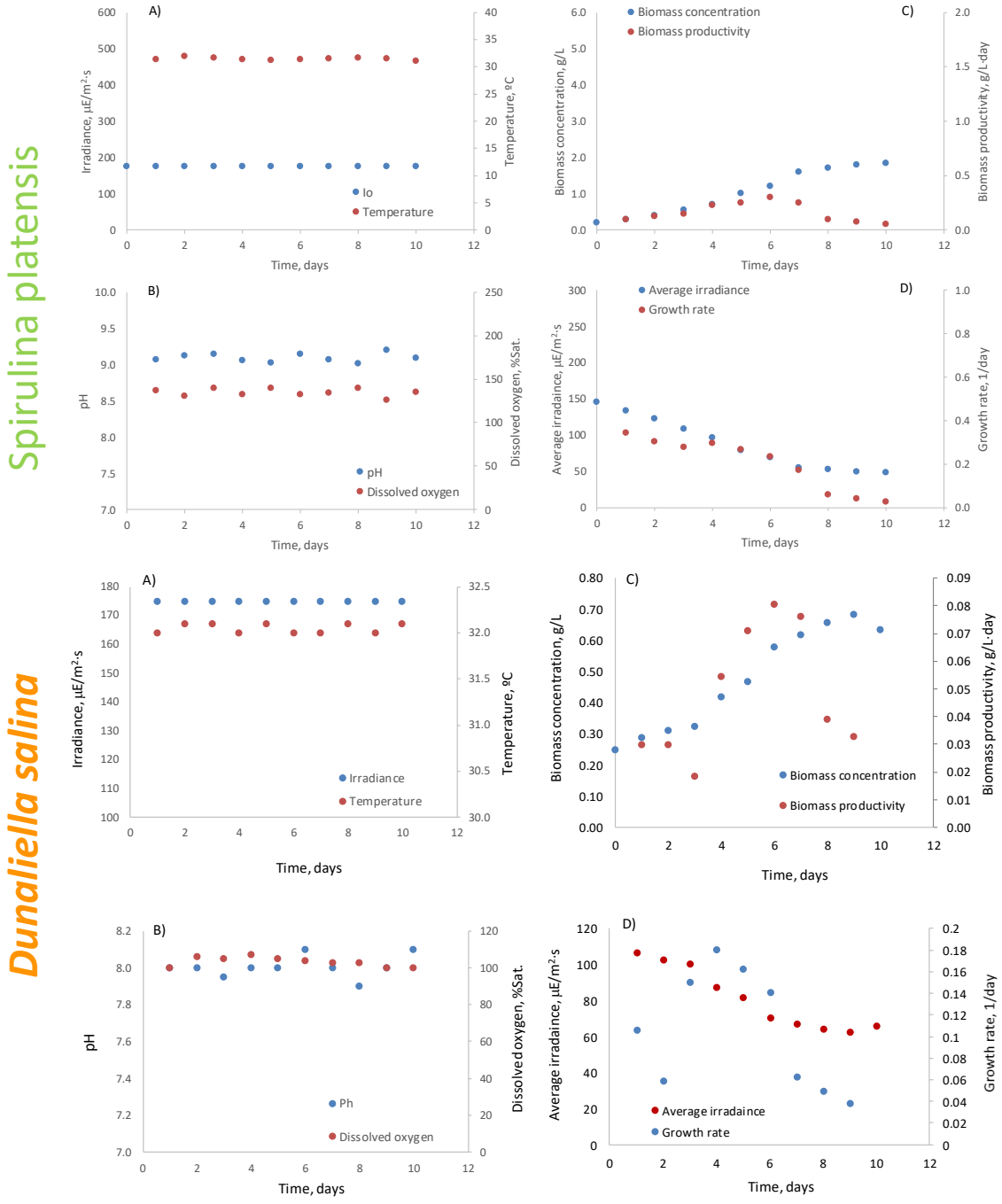


Figure 7. Resul from the batch culture of *Spirulina platensis* and *Dunaliella salina* carried out in the photobioreactor under indoor conditions. (A) Irradiance and temperature, (B) pH and dissolved oxygen, (C) Biomass concentration and productivity, (D) Average irradiance and growth rate.

4.3. Performance of the photobioreactor at different scales in outdoor conditions.

To evaluate the performance of the large-scale Fibonacci-type photobioreactor for the production of *Arthrospira platensis*, *Dunaliella salina* and *Chlorella*, semi-continuous cultures were carried most relevant results being summarized in Table 10.

Table 10. Most relevant results from semicontinuous cultures performed.

	Spirulina platensis	Dunaliella salina	Chlorella sp
Temperature and irradiance	The average solar irradiance ranged from 118 to 530 µE/m^2 s, since there are sunny days and cloudy days (Figure 8). Despite these large variations in solar irradiance, the culture temperature ranged from 30 to 34 °C; so only a narrow temperature variation was observed, and no overheating of the culture took place. The average irradiance values reduced from 250 to 40 µE/m^2 s during the batch culture and, consequently, the specific growth rate reduced from 0.55 to 0.25 1/day (Figure 8).	The solar irradiance in the light period ranged from 995 to 600 µE m^{-2} s^{-1} with only one cloudy day (Figure 8). Inside the photobioreactor, the average temperature ranged from 18.2 to 22.5 °C (Figure 8). The average irradiance daily values ranged from 100 to 200 µE/m^2 s during the semi-continuous operation of the reactor and daily values of specific growth rate ranged from 0.02 to 0.17 day^{-1} (Figure 8).	The reactor was inoculated and operated in semicontinuous mode, with 30% of the reactor being harvested when the biomass concentration achieve values higher than 1.5 g/L (Figure 8). In this period, the solar radiation was more homogeneous and intense, daily solar radiation ranging from 140 to 450 µE/m^2·s, the average daily solar radiation being 280 µE/m^2·s (Figure 8). The temperature inside the photobioreactor ranged from 23 to 32 °C, a mean value of 29 °C being determined (Figure 8).
CO_2, pH and Sat. O_2(%)	Large variations in pH and dissolved oxygen concentrations were observed due to the variations in solar irradiance - the pH ranged from 8.5 to 9.0 whereas the oxygen concentration ranged from 90 to 190%Sat. lower values being observed on cloudy days and larger values on sunny days (Figure 8).	The dissolved oxygen concentration was always below 120%Sat. Based on oxygen accumulation into the loop the oxygen production rate was measured, maximal values up to 9.2 mgO_2 L^{-1} h being estimated. The pH was maintained in the 7.5 to 8.5 range with an average of 8.0 (Figure 8).	The pH varies from 8.5 to 9.2 a mean value of 8.9 being determined (Figure 8). The dissolved oxygen concentration ranged from 59 to 130 %Sat. being measured (Figure 8). The culture was harvested three times on days 14, 20 and 27, the volumetric biomass productivity being 0.14, 0.18 and 0.16 g/L day respectively (Figure 8).

	Spirulina platensis	Dunaliella salina	Chlorella sp
Biomass concentration and productivity	Biomass concentration values up to 5.0 g/L at the end of the batch culture (10 days) were achieved, whereas biomass productivity values up to 1.1 g/L·day were obtained at the same time (10 days) (Figure 8). Data from the outdoor reactor show that, after 10 days, the stationary phase was not yet achieved, and light availability was still high enough to maintain linear growth (Figure 8).	A maximal biomass concentration 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 8). Regarding biomass productivity, considering the biomass collected when harvesting the reactor and the time between dilutions, overall values of 0.12, 0.10 and 0.11 g/ L day were measured for each one of the three steady states achieved. Analyzing the variation of biomass concentration in the three harvests were calculated, values of 0.93, 0.94 and 0.94 g/L were obtained (Figure 8). Biomass concentration achieved was three times larger than that achieved with the same strain in a raceway.	The reactor was operated in a semicontinuous mode, harvesting when the biomass concentration achieve values higher than 1 g/L, then the reactor was harvested up to three times in 30 days (days 16, 21 y 27). The average irradiance inside the cultures ranged from 43 to 226 µE/m^2·s, biomass growth rates up to 0.39 day^{-1} being measured (Figure 8). The culture was harvested three times on days 14, 20 and 27, the estimated volumetric biomass productivity being 0.25, 0.25 and 0.37 g/L day respectively (Figure 8). At these conditions, the average irradiance inside the cultures ranged from 43 to 226 µE/m^2·s during the three harvesting periods, biomass growth rates up to 0.39 day^{-1} being measured.

The adverse effect of high temperatures and dissolved oxygen concentrations, strongly reducing the performance of *Spirulina platensis* cultures in raceway reactors (Jiménez et al., 2003). In the 290 L photobioreactor, the productivity is similar to that informed in the literature; the biomass productivity in a coil reactor was 0.9 g/L day, whereas, in a near-horizontal reactor and a two-plane reactor, the productivity value was 1.5 g/L day (Tredici & Zlttelli, 1998). Only when using direct sun-oriented flat-panel reactors did the biomass productivity increase to 2.1 g/L day (Tredici & Zlttelli, 1998).

Concerning the production of *Dunaliella salina* on the reactor of 1250 L, the maximal biomass productivity was obtained at biomass concentrations ranging from 0.7 to 0.8 g/L, over 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 outdoor tubular photobioreactors in semicontinuous mode (García-González et al., 2005). On raceway reactors, values up to 0.3 g L^{-1} day^{-1} have been described (Griffiths & Harrison, 2009). These values are much lower than those reported for other microalgae strains because *Dunaliella salina* is a slow-growing strain. However, this biomass concentration is triple that previously obtained in a raceway reactor (0.3 g/ L) belonging to the Norbiotech company. In the same way for *Chlorella* (reactor of 2500 L) the measured biomass productivity was similar to values already reported for this strain, thus ranging from 0.25 to 0.37 g/L day in summertime (Hanagata et al., 1992).

New photobioreactor design allows for increased solar radiation interception; it increases the photosynthesis rate and then the CO_2 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, in the three experimented reactors. In addition, the reactors were operated in three different geographical conditions. For example, in the production of *Chlorella* sp the configuration of the reactor allows maintaining the culture conditions (temperature, pH and dissolved oxygen) in the range of recommended values for this strain (Chinnasamy et al., 2009; Daliry et al., 2017; Khalil et al., 2010; Ugwu et al., 2007).

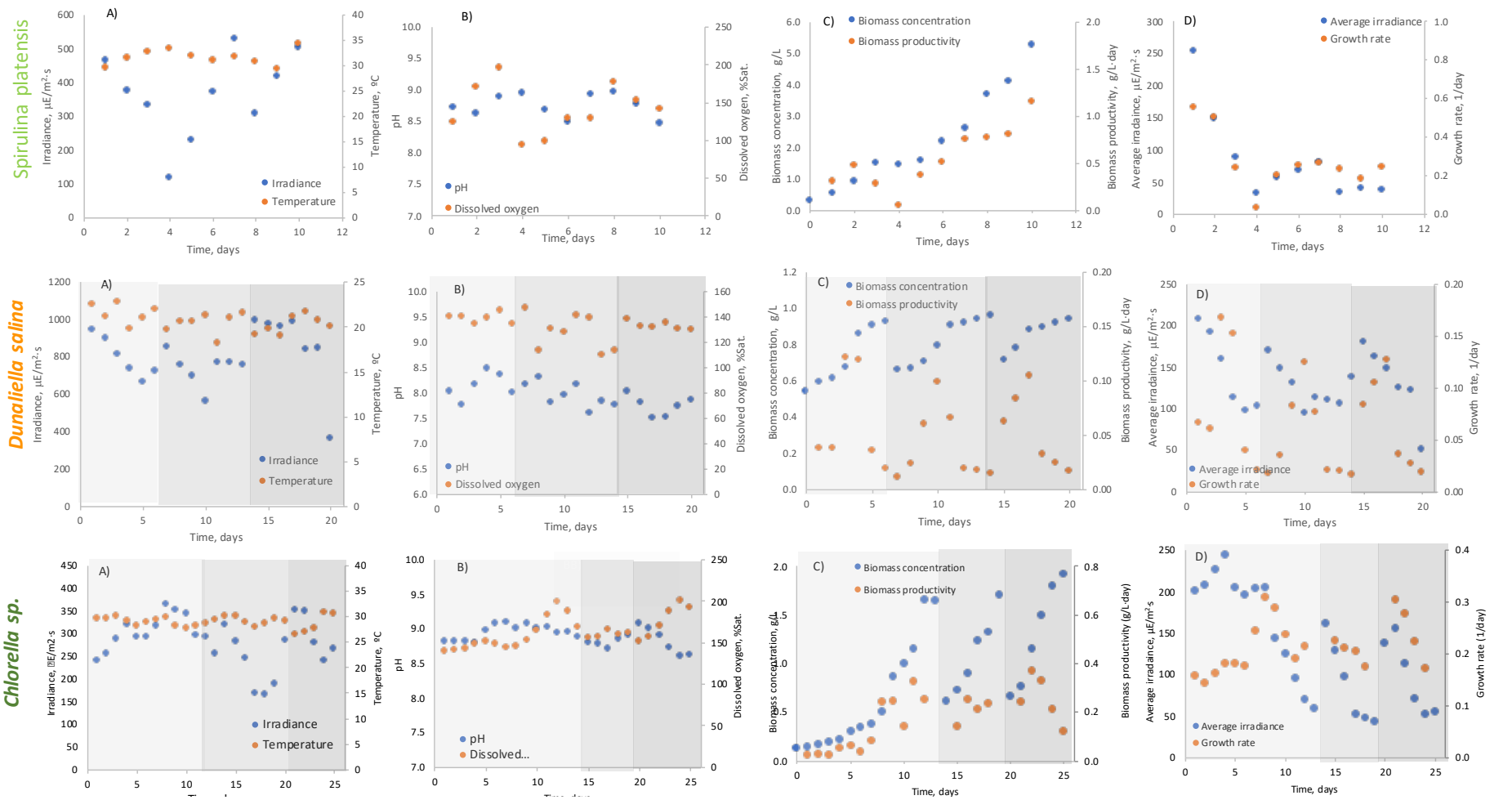


Figure 8. Data from the batch culture of *Spirulina platensis*, *Dunaliella salina* and *Chlorella* s.p. performed in the Fibonacci-type photobioreactor under outdoor conditions. (A) Irradiance and temperature, (B) pH and dissolved oxygen, (C) Biomass concentration and productivity, (D) Average irradiance and growth rate.

The photosynthetic efficiency of *Spirulina* cultures performed outdoor achieve a maximal value of 5.4%, close to the maximal theoretical value of 10% (Molina Grima et al., 1997)(Jiménez et al., 2003). Concerning the photosynthetic efficiency of *Chlorella* cultures performed on this Fibonacci-Type Photobioreactor the maximal value determined is 3.1 % (Figure 9). These data are high in comparison with other previous data, confirming the high capacity of the proposed technology to maximize the light utilization by the microalgae cells. Thus, the proposed technology was capable to maximize the light interception and providing adequate culture conditions to allow the microalgae cells to efficiently convert it into biomass (Figure 9).

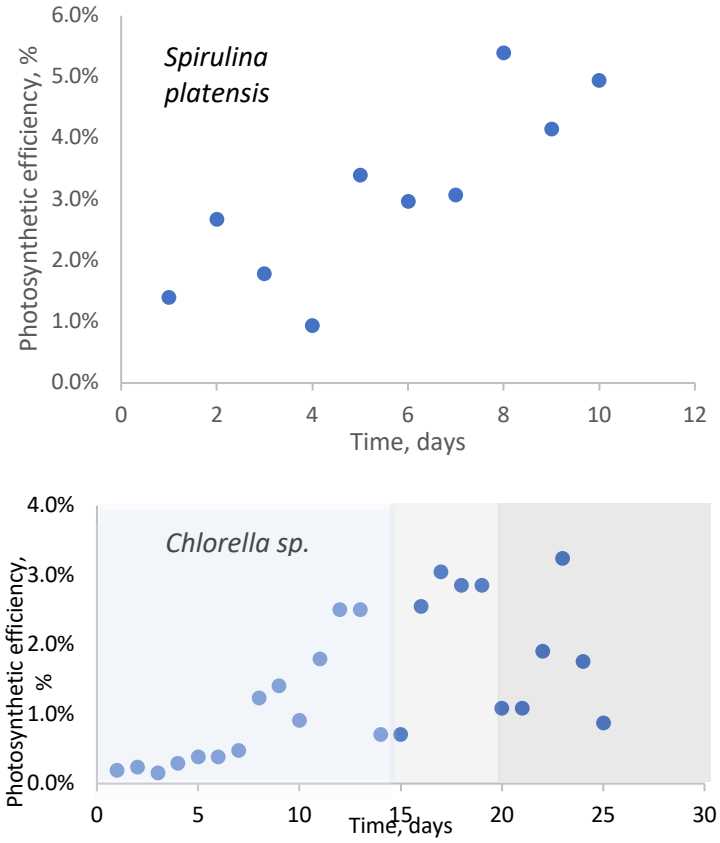


Figure 9. Photosynthetic efficiency of *Spirulina platensis* and *Chlorella* sp. culture performed in the Fibonacci-type photobioreactor under outdoor conditions.

These records confirm the reliability of using the new photobioreactor under outdoor conditions, allowing the growth rate to be maintained despite the large variation in solar irradiance registered. Thus, the proposed technology allows achieving the main goal of any reactor technology - maintaining the performance of indoor cultures under outdoor conditions.

In addition, the Fibonacci's type photobioreactor was demonstrated to be capable to produce largely different strains in outdoors conditions.

4.4. Optimization of Fibonacci-type photobioreactor

To scale up the Fibonacci-type photobioreactor is possible but it must be performed over a rationale criterion. To optimize the design of the reactor simulations were performed analysing the influence of tube diameter and biomass concentration on the maximal length of the tube and biomass productivity. In the case of the length of the tube, it must be kept below a limit to allow to maintain the dissolved oxygen concentration below the value of 200 %Sat. (F G Acién et al., 2013; Molina Grima et al., 1997; Richmond, 2004a). Simulations are performed considering tube diameters up to 0.07 to promote adequate light availability inside the reactor whereas biomass concentrations up to 4 g/L were considered as frequently found in outdoor tubular photobioreactors (F G Acién et al., 2013; Molina Grima et al., 2000). Liquid velocities of 0.30 m/s were considered to keep low the energy consumption (Gómez-Pérez et al., 2017). Increasing the speed of the photobioreactor fluid improves the degassing process, allowing the length of the tube to increase and obtain better concentrations of biomass, but in this case, the energy consumption largely increases it being a quadratic function of liquid velocity (I. Fernández et al., 2012; Molina et al., 2001).

Results show as whatever the tube diameter by increasing the biomass concentration the length of the tube increase, due to the reduction of average irradiance inside the tube, then the photosynthesis rate decreases, it allowing to enlarge the tube length while keeping the dissolved oxygen below the limit value of 200%Sat (Figure 10). The total length of the tube in the Fibonacci reactor ranged from 250 to 1600 m in the range of conditions evaluated, whereas the real length in the 2500 L reactor was 910 m for a tube diameter of 0.05 m (Table 4). Regarding biomass productivity, data shows a similar pattern whatever the tube diameter, it increases when the biomass concentration increases till it achieves a maximum at an optimal biomass concentration, then decreases (Figure 10). Similar results were also provided for tubular photobioreactors, the biomass productivity reducing when the diameter of the tube increases (Acién Fernández et al., 2001)(Torzillo et al., 2015). Maximal biomass productivity increases when reducing the tube diameter, with values ranging from 1.1 to 0.35 g/L·day when the tube diameter increases from 0.025 to 0.070 m, respectively, these values being achieved at biomass concentration reducing from 2.7 to 0.8 g/L.

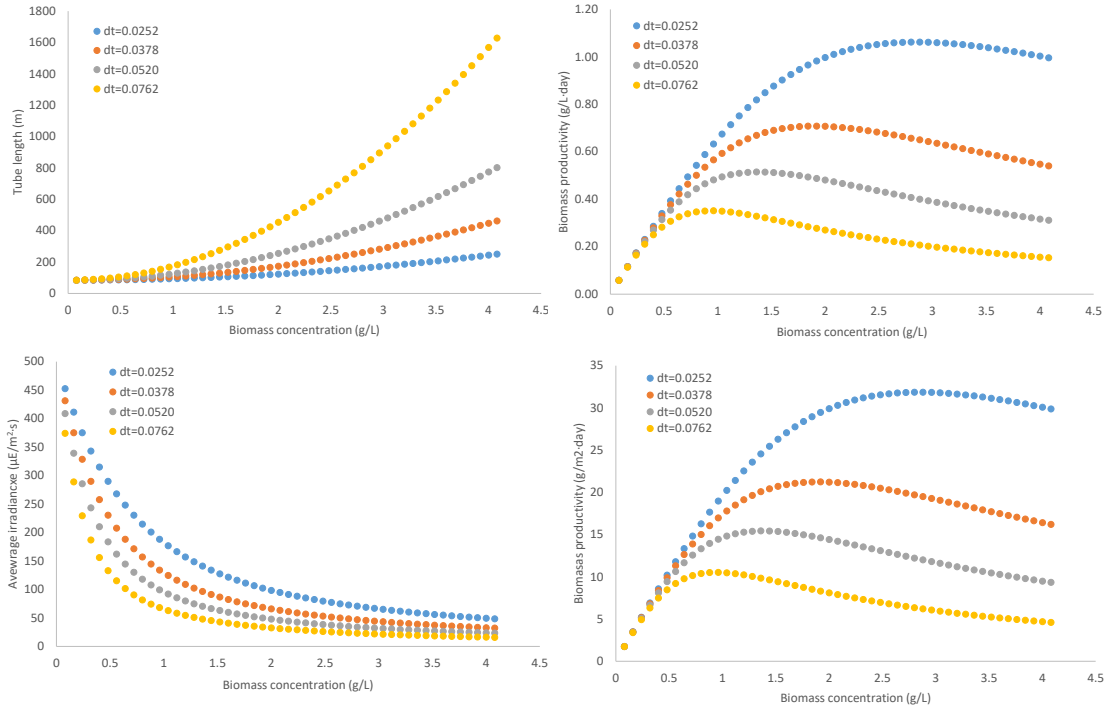


Figure 10. Parameters on the design of Fibonacci-type photobioreactor. (A) Influence of tube diameter and biomass concentration on the maximal length of the tube, (B) Variation of volumetric biomass productivity as a function of tube diameter and biomass concentration, (C) Variation of biomass productivity per surface unit as a function of tube diameter and biomass concentration.

Finally, a Fibonacci-type photobioreactor with a capacity of 5450 L is modelled, which would allow being closer to an industrial scale for the production of microalgae of commercial interest (Table 11).

Table 11. Parameters of the Fibonacci type photobioreactor scale up of 5450 L

Parameters	Value	Units
Tube diameter	0.0762	m
Tube length	910	m
Land length (L)	12	m
Land width (w)	9	m
Volume de degassing tank	1.3	m^3
Surface tube exposed to light (St)	217.8	m^2
Land occupied (S)	108.00	m^2
Volume tube (Vt)	4.15	m^3
Volume reactor (V)	5.45	m^3
V/S	50.46	L/m^2
Vt/St	19.05	L/m^2
%Vdark	0.24	
St/V	40.0	m^2/m^3

4.5. Application of microalgae in the poultry industry.

According to the management manual for layers, Hy-Line Brown commercial, the percentage of poultry egg production per week 35, is on average 92% in cage conditions. In this study, the T4 treatment achieves 84% ± 0.07 in the free-range condition and on the floor (Figure 11). Al-Ajeeli et al., (2018), report 94% egg production in layers Hy-Line Brown, in soy diet in free layers, conditions comparable to our T1 control group, which achieves 58% ± 0.01 egg production. The negative effect of thermal stress on productivity to which our treatments are exposed, mediated by the induction of oxidative stress, this difference can be explained. However, this situation can be reversed, by adding high levels of bioactive antioxidant compounds to the layer feed, allowing to reduce the damage caused by oxidative stress induced by the high environmental temperature, a situation represented by T4 (Moustafa et al., 2021). The big difference in the percentage of egg production between T4 and T1 at week 35 ($p<0.05$), could be explained by the better ability of T4 to adapt to thermal stress since T4 has more quantity of ATS 60 ppm/kg incorporated into the feed (Figure 12). For the treatments T2, T3 and T4, in the formulation of their diets have been incorporated into the food, two microalgae Spirulina and natural astaxanthin of Haematococcus pluvialis. The T1, T2 and T3 treatments showed a difference in egg production compared to the T1 control group ($p < 0.05$). However, the peak was achieved in weeks 27 and 28, with average productivity values of T4 95% ± 0.057 (W 27), T3 84% ± 0.075 (W 27), T2 79% ± 0.05 (W 28), T5 77% ± 0.14 (W 28) and T1 63% ± 0.08 (W27). Peak consistent with the Hy-Line Brown layer management manual that occurs between weeks 27 to 30, of course, with egg production values per layer, on average of 94%, much higher than the treatment T1, T2, T3, T4 and T5. In the same way, Al-Ajeeli et al., (2018) reach the spruce in week 27 with 95% egg production in the free-range diet.

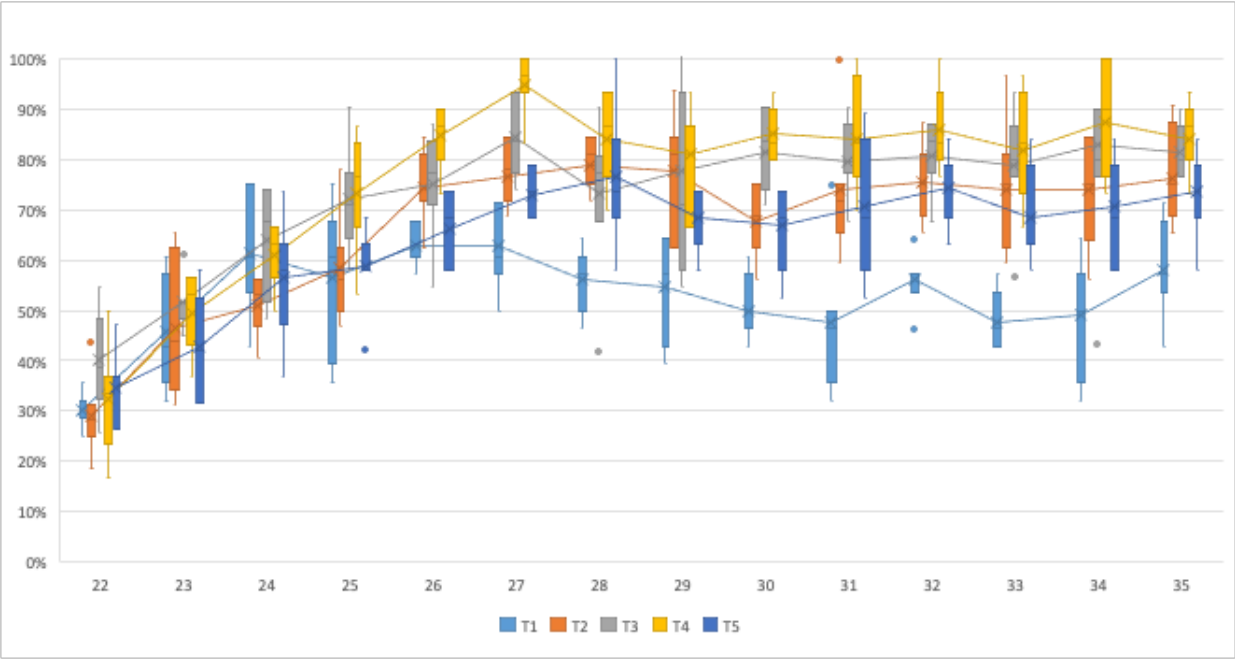


Figure 11. Weekly hen egg production: Treatment 1 (T1 control group, no microalgae), T2, T3 and T4 (Spirulina and natural astaxanthin of Haematococcus pluvialis) and T5 (only Spirulina).

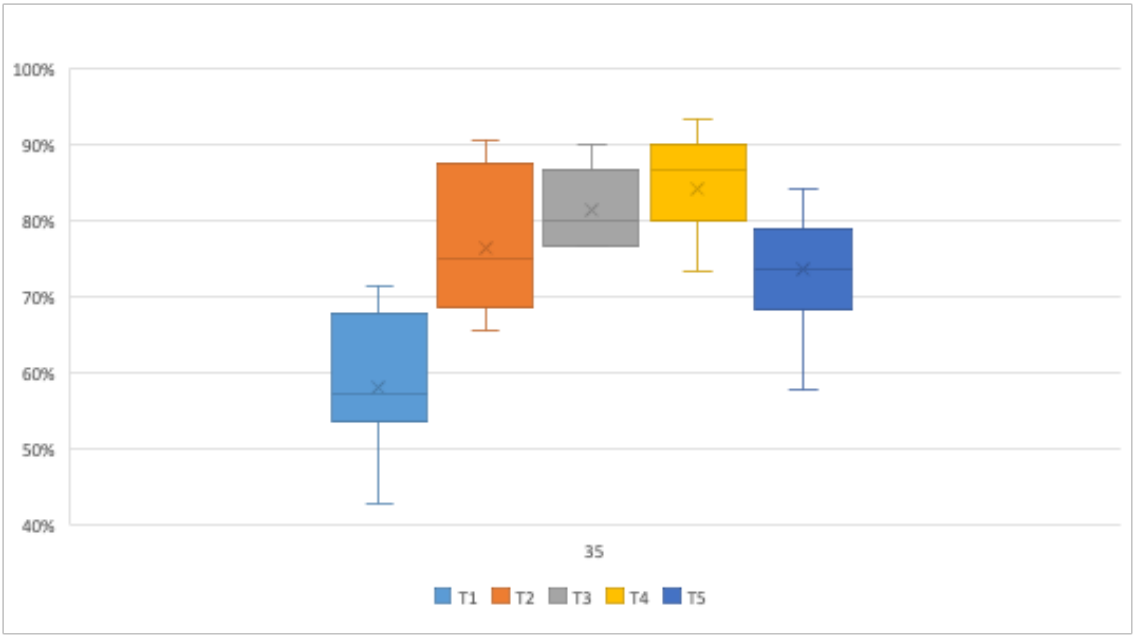


Figure 12. Percentage of egg production in week 35: Treatment 1 (T1 control group, no microalgae), T2, T3 and T4 (*Spirulina* and natural astaxanthin of *Haematococcus pluvialis*) and T5 (only *Spirulina*)

The evolution of live weight gain is not significant in laying hens between weeks 22 and 32 (p<0,05). The differences begin to occur between weeks 34 and 36 (P < 0.05). Registering in the week 36 average pesos in the layers of: T4 2,21 Kg ± 0,061; T3 2,1 Kg ± 0,059; T2 2,1 Kg ± 0,03; T5 1. 99 Kg ± 0,048 y T1 1,88 Kg ± 0,022 (Figure 13). According to the manual Hy-Line Brown body wight (W36) is from 1.92 Kg. In addition, it is highlighted that the T1 control group from week 34 to 36 lost weight (P < 0.05), from 1.95 kg ± 0.43 to 1.88 Kg ± 0.0.2. The situation is similar to egg production by hens, explained by stress. It is noted that the Indeed the incorporation of microalgae into the feed of poultry producing egg and meat, increase the body weight (Kang et al., 2013)(Ragab & Fathi, 2018)(Abdelnour et al., 2019)(El-Bahr et al., 2020) (Souza et al., 2020) (Souza et al., 2020).

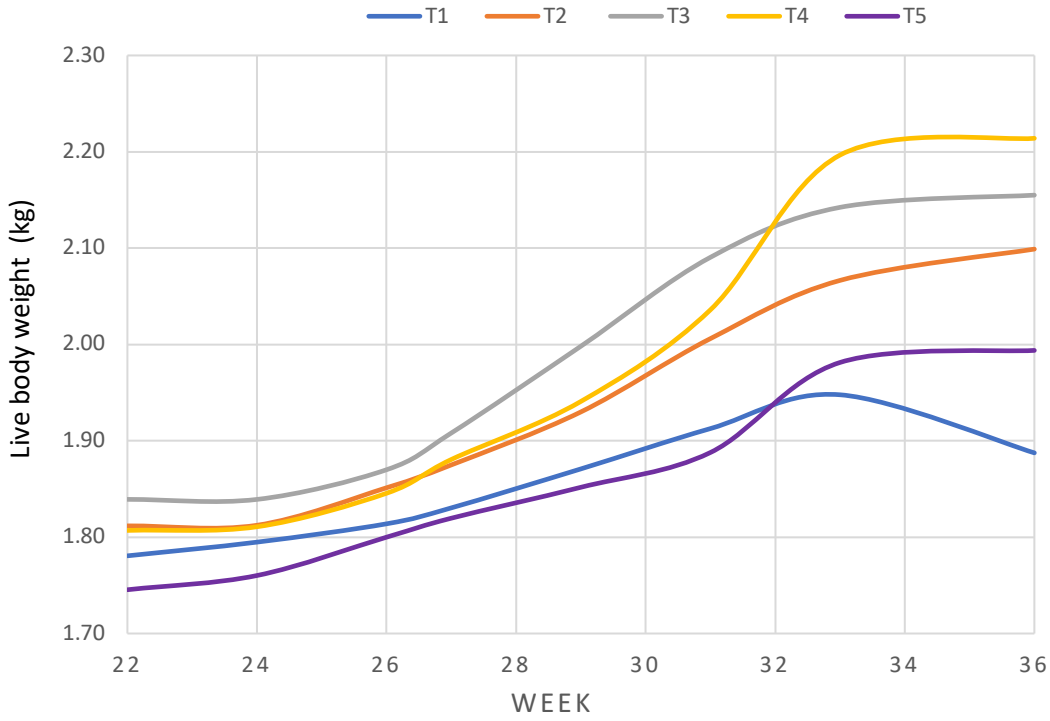


Figure 13. Average evolution of live weight gain total (Kg) between the different treatments and the control group.

Concerning average growth rates, there are no differences until week 31 (GR 22-31) (P>0.05). However, at week 36 there are differences in growth rates between treatments (p < 0.05), whose average values are GR T4 0.05 ± 0.011; T3 0.04 ± 0.015; T2 0.036 ± 0.013; T5 0.03 ± 0.011 and T1 0.01 ± 0.006. In the study period, all treatments experienced weight growth, except for the control group (T1) which achieved it until week 32, from there it decayed (Figure 14). Microalgae such as *Chlorella* and *Spirulina* improve growth factors in egg and meat

producing poultry, here the treatments that have microalgae T2, T3, T4 and T5, show higher growth rates than the T1 control group ($p < 0.05$). It is observed that diets containing higher amounts of astaxanthin achieve higher growth rates, remaining in the last position, T5 which only presents 1% of *Spirulina*. There is evidence that by supplying microalgae Chlorella and Spirulina to feed they improve growth factors in birds (Kang et al., 2013) (Kotrbáček et al., 2015) (Al-Ajeeli et al., 2018)(Ragab & Fathi, 2018)(El-Bahr et al., 2020)(Moustafa et al., 2021). To date, no studies have been reported, where two microalgae (*Spirulina* and *Haematococcus pluvialis*) are incorporated into the bird's feed, as is this study.

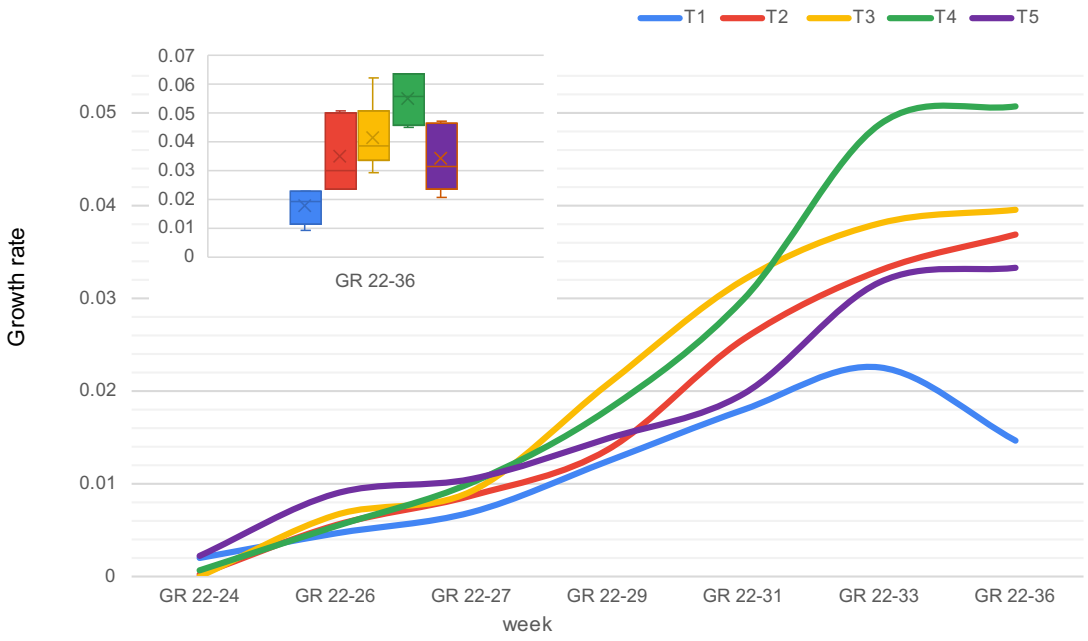
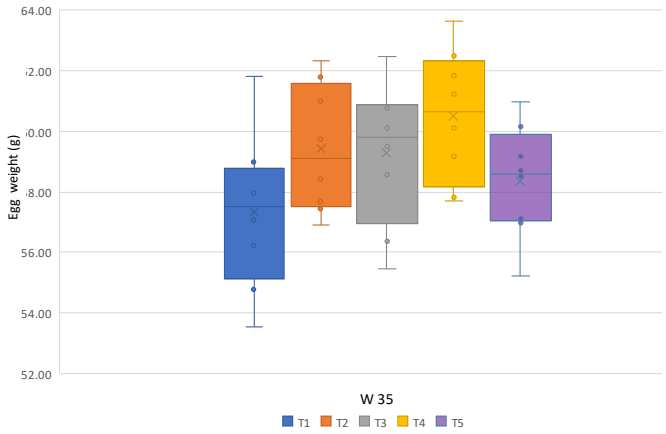


Figure 14. Average evolution of the growth rate between the different treatments and the control group.

The variables that measure the quality of the egg until week 35, do not present a difference between the different treatments ($p > 0.05$), showing the following average weights for treatments T4 60.5 g ± 2.18; T2 59.4 g ± 4.43; T3 59.3 g ± 5.6; T5 58.3 g ± 3.4 and T1 57.3 g ± 2.57 (Figure 15A). According to the manual Hy-Line Brown Egg weight (W36) is from 62 g. The resistance of the Eggshell was 4.4 Kgf. This variable did not present significant differences between the treatments ($p > 0.05$), being their averages by treatments T4 5.2 Kgf ± 0.73; T3 5.05 Kgf ± 1.29; T2 4.47 Kgf ± 1.69; T5 4.96 Kgf ± 0.30 and T1 5.0 Kgf ± 0.30 (Figure 15B).

A)



B)

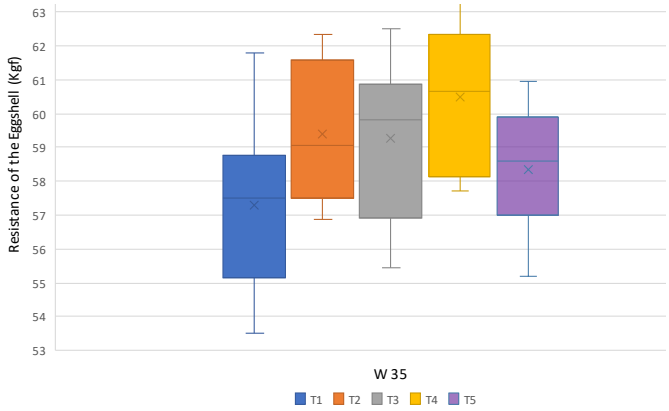


Figure 15. A) Egg weight (g) in the week 35 and B) Resistance of the Eggshell (Kgf): Treatment 1 (control group T1, without microalgae), T2, T3 y T4 (*Spirulina* and astaxanthin) y T5 (only *Spirulina*).

Equally the eggshell thickness at week 36 between treatments, no differences significative ($p > 0.05$) (Figure 16). Thickness and pigmentation are directly or supposedly related to the strength of the eggshell (Gosler et al., 2005). In addition, other factors are those that interfere with the mineralization and quality of the shell, including environmental and nutritional factors, as well as the state of health and well-being of commercial hens (Bozkurt et al., 2012). In this case, the birds are in a free state, a situation that favored maintaining the quality of the shell, despite also being subjected to thermal stress.

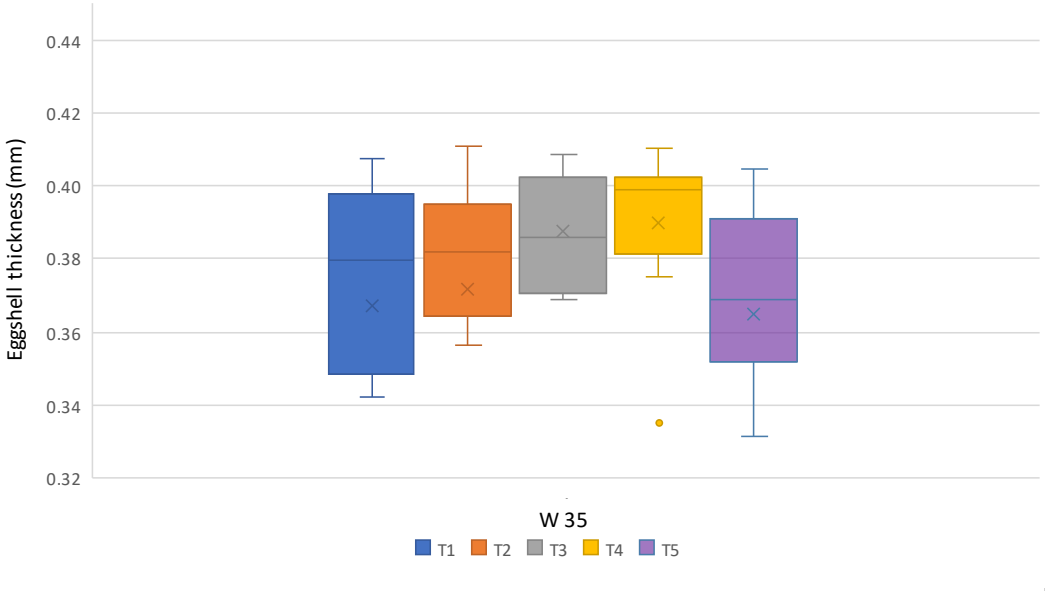


Figure 16. Estimates Eggshell thickness at week 35, between treatments (T1, T2, T3 y T4 and T5)

Estimates of the colour of the egg yolk at week 35 (DSM Yolk Color Fan), show that within the colour scale the T4 achieves the highest value being 40% of the buds examined on scale 14 and 60% on scale 13, in descending form follows the T3 treatment 70% on scale 12 and 30% on scale 13, for T2 its values are 80% in scale 12 and 20% in scale 11, and for T5 60% in T11 and 40% in scale 10 (Figure 17). Finally, the T1 control group only achieves 50% in 4 and 50% on scale 3. For T5 treatment that only has Spirulina in the feed-in 1%, Omri et al., (2019), found a significant improvement in egg yolk colour in response to spirulina supplementation. In this study, T5 achieves an increase in the colour scale 2.75 times compared to the control group, on the other hand, T4 has two microalgae in the feed (Spirulina and *Haematococcus pluvialis*) achieving an increase concerning the control of 3.5 times. As Spirulina is added and gradually the amounts of natural astaxanthin in the feed of the hens are increased, the colour of the egg yolk increases reaching a maximum of 14, under conditions of thermal shock.

Walker et al., (2012), showed that feeding with 1.35% astaxanthin (from algae), in Hy-Line W36, did not affect production yield or egg quality except for egg yolk colour. However, Heng et al., (2021), valuate the effects of astaxanthin from *Haematococcus pluvialis* on production performance, egg quality, antioxidant enzyme activity and free radical scavenging ability. Incorporated into the feed 0, 20, 40, 80, or 160 mg/kg ASTA for 4 weeks, values similar to our study. Their results show no significant effects were observed on egg weight, feed consumption, feed efficiency, laying rate, Haugh unit, or eggshell strength. The addition of *Spirulina* makes a difference concerning the laying rate. In addition, evidence that yolk colour

darkened linearly with an increasing dose of astaxanthin. There are other experiences of using microalgae, for example, in *Schizochytrium powder*, the inclusion of 0.5% of *Schizochytrium power* in the diet of the layers improved the colour of the egg yolk (da Costa Santana et al., 2021).

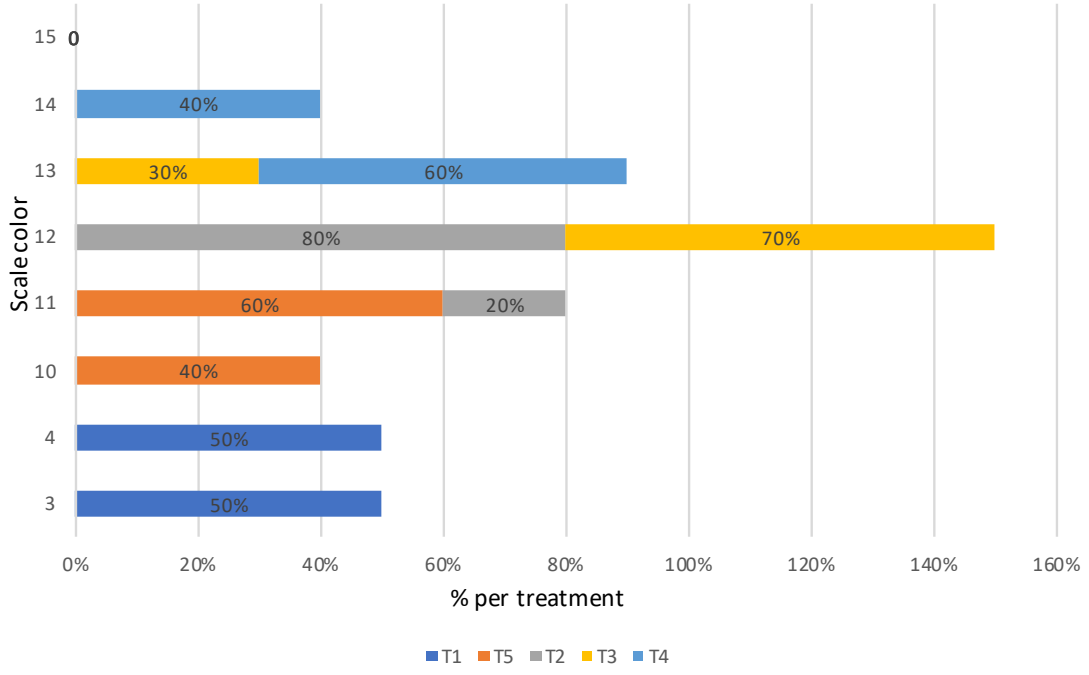


Figure 17. Estimates Eggshell thickness at week 36, between treatments (T1, T2, T3 y T4 and T5)

Concerning the presence of carotenoids in fresh yolk at week 35, the results show that there are significant differences between all treatments ($p < 0.05$). This behaviour is similar to that obtained when evaluating the colour of the yolk. There is a high correlation between carotenoid content and yolk colour (Valcu et al., 2020) (Figure 18). The T4 treatment achieves 270% ± 37% more carotenoids compared to the T1 control group, followed by T3 with a 198%± 40%, T2 achieves a 174% ± 28% and the T5 group that has the addition in the feed 1% of Spirulina achieves 158% ± 22% concerning the control. By gradually adding in the treatments T2, T3 and T4 *Spirulina* and natural astaxanthin of *Haematococcus pluvialis,* the amounts of carotenoids in the egg yolk increase, reaching a maximum in the treatment T4. Other studies show that the addition of microalgae in low percentages increases carotenoid contents in the egg yolk (L. Gouveia et al., 1996)(Kotrbácek et al., 2013) (Goula et al., 2017)(da Costa Santana et al., 2021).

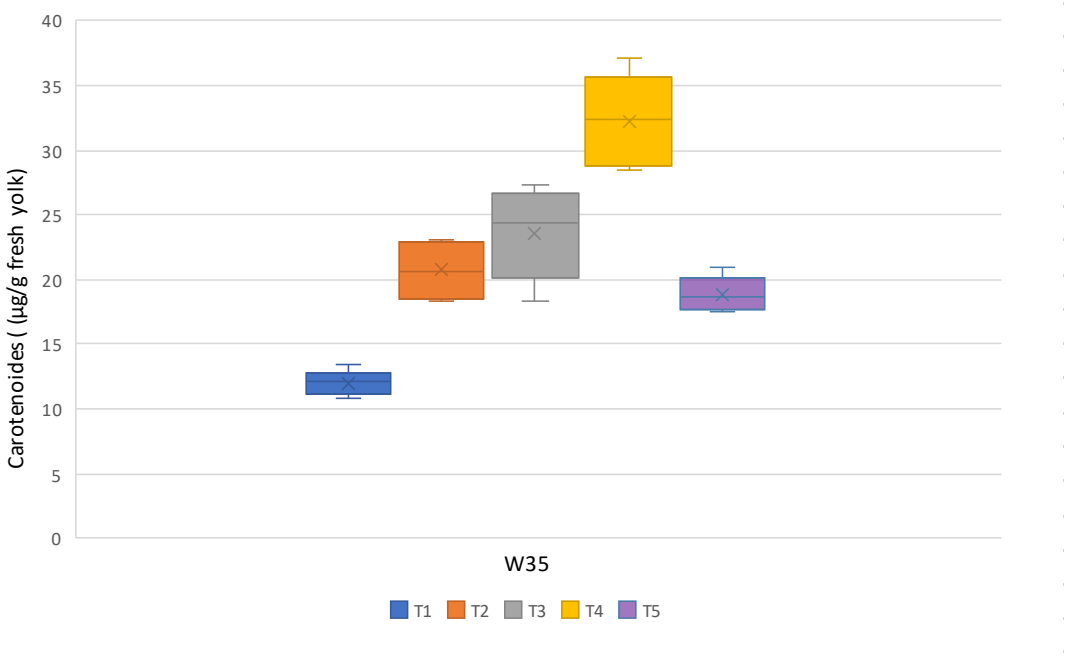


Figure 18. Estimates of carotenoids in fresh yolk at week 36, between treatments (T1, T2, T3 y T4 and T5)

5. CONCLUSIONS

5.1. Fibonacci-type tubular photobioreactor for the production of microalgae

In this work, a new tubular photobioreactor design following the Fibonacci equation has been proposed. This new concept for the tubular photobioreactor has been evaluated at two scales, under indoor and outdoor conditions, with both maintaining the same aspect ratio. The results confirm that the proposed design allows up to a 1.4-times increase in intercepted solar radiation compared to that received on a horizontal surface while providing optimal culture conditions growth - moderate temperature, adequate pH and low dissolved oxygen concentration. During the production of *Spirulina platensis* specific growth rates up to 0.55 1/day and photosynthetic efficiencies up to 5.4% was measured outdoors, thus confirming the reliability of the proposed technology for producing microalgae. Nonetheless, the design and construction of this new photobioreactor type must still be improved; that said, this work demonstrates the potential of this new concept in improving microalgae culture productivity; a major challenge for the development of microalgae technology related to the industry.

5.2. Scale-up of a Fibonacci-type photobioreactor

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 capturing 1.6 times more solar radiation than the horizontal surface while providing optimal conditions for *Dunaliella salina* production without the need for cooling, it also allows maximal efficiency of CO_2 utilization and avoids 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 m^{-2} day^{-1}, 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* on a commercial scale.

5.3. Large scale development of a Fibonacci-type photobioreactor

The new concept of Fibonacci-type tubular photobioreactor has allowed the production of *Chlorella* sp. at a scale of 2500 L, favouring optimal environmental conditions to produce this microalga. Concerning culture conditions such as temperature, pH and dissolved oxygen it was possible to adequately control them, doing unnecessary to spend energy on cooling and injecting air bubbles to remove the excess oxygen. However, the photobioreactor scaled to 2500 L did not achieve high efficiency, as its design parameters were not optimal. This unfavourable situation did not prevent the adjustment of the design parameters of the Fibonacci-type

photobioreactor, since it is possible to manage its geometry in length, width and height, and to make changes in the diameter tube. All this adjustment was done using mathematical modelling that allowed to obtain optimal design parameters and thus overcomes one of the great problems that the tubular photobioreactors present, which is their limited possibility of being extended. This modelling, in turn, allows considering the physiological needs of the microalgae, especially the photosynthesis rate. The new design parameters of the Fibonacci-type photobioreactor allow to scale it up to 5450 L. The flexibility in the design of the photobioreactor is due to the geometric concept of the Fibonacci spiral.

5.4. Application of microalgae in the poultry industry

The new concept of Fibonacci-type tubular photobioreactor has allowed the production of shape efficient and quality microalgae. On the other hand, tubular photobioreactors are called to produce high-value substances such as pigments. But it is not enough to be efficient in the production of microalgae. As we know the market for microalgae is small, so the need to test microalgae in larger markets. One of the large markets is the Avicola industry. In this context, the combination of two microalgae in egg production was tested. It's doable that combining *Spirulina Platensis* and *Haematococcus pluvialis,* and incorporating them into the diet of laying hens, have positive effects on the improving egg quality. That is increasing the colour of the yolk on the scale “DSM Yolk Color Fan” and increasing carotenoids in the egg yolk. In addition, better productive performance is evidenced in the live body weight gain y increase in the percentage of egg production.

6. LIST OF CONTRIBUTIONS

6.1. Publications

Díaz, J. P., Inostroza, C., & Acién Fernández, F. G. (2019). Fibonacci-type tubular photobioreactor for the production of microalgae. Process Biochemistry, 86(April), 1–8. https://doi.org/10.1016/j.procbio.2019.08.008

Díaz, J. P., Inostroza, C., & Acién, F. G. (2021). Scale-up of a Fibonacci-Type Photobioreactor for the Production of *Dunaliella salina*. *Applied Biochemistry and Biotechnology*, *193*(1), 188–204. https://doi.org/10.1007/s12010-020-03410-x

Díaz, J. P., Inostroza, C., & Acién Fernández, F. G. (2022). Scale-up of a Fibonacci-type photobioreactor for the production of *Chlorella sp*. *Process Biochemistry*. Submitted article

6.2. Communication in congress, course, conference, symposium,..

*** Indicate the Event Type: Congress**

* Indicate the Event Type: VII CONGRESO NACIONAL DE ACUICULTURA CHILE
*Title of the Paper or Communication: Design of a photobioreactor for the super-intensive production of *Chlorella* microalgae biomass in northern Chile
* Authors of the Paper or Communication: Juan Pablo Díaz, German Bueno y Walton Cabrera
* Center, Institute or University where the activity has been held: Universidad Arturo Prat
*Localidad donde se ha celebrado la actividad: ARICA
* Country where the activity was held: CHILE
* Start Date of the activity: 11/09/2019
* End date of the activity: 14/09/2018

***Indicate the Event Type: Simposio**

*Indicate the Event Type: IX SIMPOSIO DE INVESTIGACIÓN EN CIENCIAS EXPERIMENTALES 2020
* Title of the Paper or Communication: New design of a photobioreactor for the massive production of microalgae, named Fibonacci-type
*Authors of the Paper or Communication: J.P. Díaz, C. Inostroza, y F.G. Acién
*Center, Institute or University where the activity has been held: Universidad de Almería
*Locality where the activity has been held: Almería
*Country where the activity was held: España
*Start Date of the activity: 13/11/2020
*End date of the activity: 13/11/2020

6.3. Articles published in books of abstracts of congress

Díaz, J. P., Inostroza, C., & Acién, F. G. (2021). NEW DESIGN OF A PHOTOBIOREACTOR FOR THE MASSIVE PRODUCTION OF MICROALGAE, NAMED FIBONACCI-TYPE. In J. L. López, I. Fernández, M.C. Cerón, J.F. Mañas and J. Moreno, IX Simposio de Investigación en Ciencias Experimentales, Universidad de Almería. http://www2.ual.es/isimpos/

Díaz, J. P., Bueno, G., & Cabrera, C. (2018). Diseño de un fotobiorreactor para la producción superintensiva de biomasa de microalgas *Chlorella*, en el norte de Chile. In Sociedad Chilena de Acuicultura, VII Congreso Nacional de Acuicultura: "Por la Sustentabilidad de la Acuicultura en Zonas Áridas", Universidad Arturo Prat, Arica.

6.4. Awarded projects

Project name: Happy Laying Hens: Microalgal biotechnological development to enhance the immune system in hens, producing eggs with antioxidants, in the province of Loa.

*Participating entities: Universidad Arturo Prat, Universidad de Chile, Universidad de Almería, Agricultores de Calama y empresas biotecnológicas
*Project Managers: Juan Pablo Díaz Vega
*Type of Participation: Manager
*Country Project: Chile
*Project Start Date: 01/04/2020
*Project Start Date: 30/09/2021

*Justificantes Participación: Code BIP 400013473-0

Project name: Biotechnological development and use of *chlorella* microalgae as a natural chelator and protective power against contamination by polymetals (lead)

*Participating entities: Universidad Arturo Prat, Universidad de Almería and Solarium Biotechnology S.A.
*Project Managers: Juan Pablo Díaz Vega
*Type of Participation: Manager
*Country Project: Chile
*Project Start Date: 01/03/2022
*Project Start Date: 30/11/2023
*Justificantes Participación: Code BIP 40037400-0

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