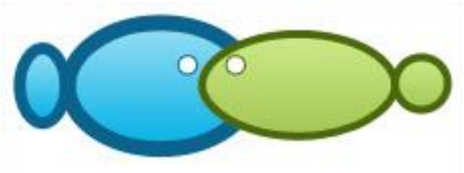

Population growth of *Chlorella* sp. in three types of tubular photobioreactors, under laboratory conditions

[1]Yeni del S. Matabanchoy-Mesias, [1]Yésica A. Rodríguez-Caicedo, [2]Marco A. Imués-Figueroa

[1] Program of Aquaculture Production Engineering, Faculty of Animal Sciences, University of Nariño, Pasto, Colombia; [2] Department of Hydrobiological Resources, Faculty of Animal Sciences, University of Nariño, Pasto, Colombia. Corresponding author: M. A. Imués-Figueroa, marcoi@udenar.edu.co

Abstract. Microalgae require certain physical and chemical conditions of the culture water and adequate growth nutrients availability to develop. Therefore, the growth curve of *Chlorella* sp. microalgae cultivated in 3 types of tubular photobioreactors (helical, conical and coil) was evaluated, under laboratory conditions with artificial light, for which a randomized complete block design with sub-sampling was used, consisting of 3 treatments corresponding to 3 types of tubular photobioreactors, with 4 blocks corresponding to 4 runs at different times. Variables such as: algal density, simple growth rate, and control variables dissolved oxygen, pH, temperature, carbon dioxide, airflow, flow, and fluid velocity were evaluated. Growth curves were estimated using a potential multiplicative model of form $y=ax^b$, using the Statgraphics Centurion program. The results showed that the best density was achieved by the conical tubular photobioreactor (CTPHBR), with maximum cell growth ($23.666\times10^6\pm887,568$ cell mL^{-1}) at 12 days of culture and a simple growth rate (SGR) of 0.72±0.31% $hour^{-1}$; the helical tubular photobioreactor (HTPHBR) reached the highest cell density at 9 days of culture ($18.937\times10^6\pm923,592$ cell mL^{-1}) and an SGR of 3.0±0.18% $hour^{-1}$; the coil tubular photobioreactor (CLTPHBR) reached cell density of $5.038\times10^6\pm939,354$ cells mL^{-1} at 6 days of culture, with an SGR of 1.90±0.24% $hour^{-1}$, whose significant differences ($p<0.05$) suggest that HTPHBR and CTPHBR provide better conditions for the exponential growth of *Chlorella* sp. The results of chlorophyll-a in different treatments showed that the highest concentration was obtained by HTPHBR (1.88±0.26 mg m^{-3}), followed by CTPHBR (1.87±50.20 mg m^{-3}), and the lowest concentration was found in the CLTPHBR (1.42±0.27 mg m^{-3}) with significant differences ($p<0.05$) between the latter and the two others.
Key Words: aquaculture, cyanobacteria, algal culture, photobioreactor, growth curve.

Introduction. In many species of fish and crustaceans, the larvae do not actively feed; they survive by consuming the reserve of their yolk sac and, shortly before full absorption, they begin to consume natural foods, usually microalgae and rotifers. Those reasons have led to studying the plankton, as it represents the first link in the food chain (Gómez 2007; Prieto et al 2005), mainly made up of microalgae (Vera et al 2009; Ordoñez 2017), whose reproduction occurs mostly by cell division, in most cases binary. Growth is rapid when they are inoculated in a closed culture medium and maintained in suitable conditions, which changes with the age of the crop, varying the population growth rate in different phases of growth, which describes the biomass change (Arredondo-Vega 2017).

Currently, the worldwide production of microalgae is destined to different applications of high added value, due to their content of vitamins, pigments, proteins, essential lipids, carbohydrates and minerals. Microalgae are mainly used assource of energy, biocatalysts for CO_2 biomitigation from combustion gas, raw material in human food, animal food and cosmetic industries and in the wastewater treatment process (Toledo-Cervantes et al 2018). As much as 30% of the microalgae volume goes to animal consumption. Species of *Chlorella*, *Scenedesmus* and *Spirulina* genus are beneficial for animals, improving immune response, infertility and weight control (Brennan & Owende

2010). In aquaculture, they are the primary source of the feeding of filtering mollusks and larval states of fish and crustaceans (Henández & Labbe 2014). *Chlorella* sp. is a good source of quality proteins, in addition its ease of cultivation by growing in highly selective environments, being immune to the contamination of other algae and protozoa (Toledo-Cervantes et al 2018).

Despite their simplicity and wide distribution, microalgae require favorable conditions, with limiting ecological factors such as temperature, dissolved oxygen, luminosity, and the concentration of certain nutrients that are currently poorly controlled environmentally (Sandoval-Riofrío 2013; Bertoldi et al 2006). *Chlorella* crops constitute a technology of medium complexity pilot plants, at a temperature between 18 and 25°C, pH of 6-8, luminosity of 2,000-4,000 lux, and nitrogen concentration around 10-18% (Plasencia 2012). The extraction, identification and quantification of pigments, such as chlorophyll, are carried out by different methods. Microalgae are also used as additives in pharmaceutical formulas and processed foods (Rivera & Tchaikina 2014).

In the last years, studies have been carried out to determine the population growth of microalgae within structures with a wide variety of designs in open or in closed systems, called photobioreactors (PHBR), with little or no contact with the external environment (Hernández & Labbe 2014). PHBRs are considered as an alternative to achieve large biomass in smaller areas, designed to overcome some of the main problems associated with open production systems, in particular the risk of contamination, via monocultures (Brennan & Owende 2010). The PHBRs are transparent closed systems of plastic or glass and with geometries of various types (flat plate, tubular, suspended bags, column), which depend on local conditions, the product to be obtained, and the economic specifications of the project (Barry et al 2016). In tubular PHBR, higher productivity is achieved, mainly because they improve the efficiency of photosynthesis, maintaining a monoculture is easier and they enhance the product purity, making it suitable for the food industry. The choice and design of the reactor collect the light required to maximize the microalga organism's vital function (Toledo-Cervantes et al 2018).

The present study was developed in order to evaluate the growth curve of the *Chlorella* sp. microalgae in three types of tubular photobioreactors (helical, conical, coil) with artificial light, under laboratory conditions and determine the chlorophyll-a concentration in the different PHBR.

Material and Method

Location, laboratory, and equipment. The experiments were carried out in the Aquaculture Laboratories of the University of Nariño, located northwest of the city of San Juan de Pasto (Colombia), at a height of 2,486 m above sea level and an average ambient temperature of 13.8°C (Imués-Figueroa et al 2018).

For the scaling of the microalgae culture, a 0.5 m^3 cabin was used, with three-level shelving and three 30 Wats cold light lamps, where the strains and massive small-scale cultivation were located. The primary stock of algae in small volumes (10 to 200 mL) was established in the cabin; in the area of mass culture, primary crops were developed up to 3 L volumes to obtain the inoculums sown in the PHBR. The vessels were provided with aeration using a 125 W Hailea blower.

In the space of 7.02 m^2, in the crops area, the three tubular PHBRs were available within a laboratory with a surface area of 84.6 m^2. The tubular photobioreactors (TPHBRs) have a volume of 45 L, built with 56 m of one-inch glass hose, rolled on welded mesh with a diameter of 0.4 m for the TPHBR; the CTPHBR is 1.4 m in diameter in the lower section and 0.2 m in diameter in the upper section. The useful height is 1.53 and 1.04 m, respectively and total height of 1.53 m; the CLTPHBR has 48.64 m of hose and 24 elbows of 180° PVC with 30 cm in length; the tube surrounds a flat support of 2.15 m long and 0.9 m wide forming a coil arranged on the wall, guaranteeing an equal height for all. Each TPHBR has six 22W white light LEDT8 lamps providing 2,400 lumens.

After the assembly of TPHBRs, those were filled with water previously settled and dechlorinated; the pressure was introduced with air, including an airlift pump to propel

the fluid into the dark phase. This device is of a 20 L plastic container, with a narrow mouth, measuring 0.51 m in height, and 0.25 m in diameter, being located on the support of 1.60 m tall, in such a manner that the liquid can continue by gravity towards the photic zone (Figure 1).

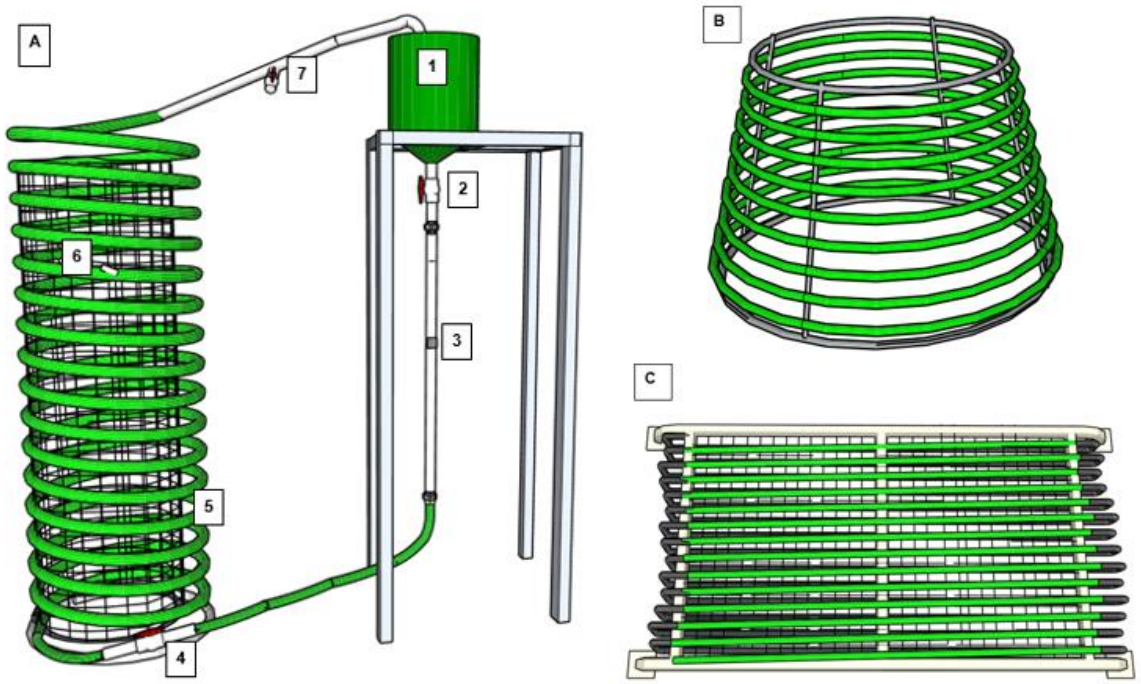

Figure 1. Models of tubular photobioreactors developed for research. A: HTPHBR, B: CTPHBR, C: CLTPHBR; 1: Dark phase reservoir, 2: Stopcock, 3: Flowmeter, 4: Stop valve and CO_2 addition site, 5: Fluid with illumination 24:24 hours of photoperiod, 6: Airlift insertion, 7: Spanner sample extraction step (original).

Biological material. The strain of *Chlorella* sp. came from the same Aquaculture Laboratories of the University of Nariño, where it was kept pure. The microalgae were replicated and raised to a volumen of 3 L. Inoculum was planting in the TPHBRs, with a supply of F/2 of Guillard nutrient in a proportion of 1 mL L^{-1} of fluid.

Protocol for sowing microalgae. Based on the protocol recommended by Helm et al (2006), a specific protocol for the planting of microalgae was developed. The in-vitro growth procedure was developed inside a cabin sterilized with UV light. Materials (tubes, pipette tips, beaker) were sterilized in a 24 L electric autoclave. Surfaces of the inoculation site in the culture vessels (10-200-3,000 mL) were cleaned with 85% ethanol, in the heat of a lighter. Nutrients were added with automatic micropipettes of 1-100 µL and 100-1,000 µL, keeping the lighter lit at close range. The 3,000 mL cultures were started with an inoculum of 310,833±70,383 cell mL^{-1} and a simple growth rate of 14.79% day^{-1}.

The basic parameters were quantified within the laboratory: temperature (24.08±0.73°C), using a digital system and heating thermostats; dissolved oxygen (OD) (4.21±0.55 mg L^{-1}), with controlled aeration supply; pH (7.72±0.41) monitored with digital system; CO_2 supply by solenoid valve; illumination with cold light lamps (2,321.18±392.04 lumens). The cultures were replicated to a volume of 3 L at a concentration of 4.133×10^6±722,684 cell mL^{-1}, when the inoculum was sown in the TPHBR. Four runs were made at different times, for cultivation of the microalgae.

Cell count. A Neubauer chamber was used to perform the counts. It was calculated from the data obtained following the method described by Sipaúba-Tavares & Rocha (2003), to determine the density daily, three times per day.

Determination of chlorophyll-a. The microalgae lyophilization pretreatment and the cell lysis (cellular maceration in mortar cup maintaining low temperatures with ice) were performed directly on the fresh culture every three days, following the American Public Health Association's (APHA) harvesting protocol (Huarachi et al 2013), where the centrifuge speed was modified at 4,500 rpm for ten minutes. The resulting dried pellets were analyzed by spectrophotometry.

Experimental design and statistical analysis. The one-way ANOVA for the completely randomized design (CRD) with subsampling showed significant differences ($p<0.05$) between runs, therefore the two-way ANOVA was performed for a randomized complete block design (RCBD) with sub-sampling, in order to reduce the experimental error. The RCBD was made up of three treatments corresponding to the types of TPHBR, and four blocks, consisting of runs at different times. Separate analyzes and measurements in each sampling, for treatments configuration within the blocks, were considered as subsamplings. Statistical analyses were performed using the Statgraphics Centurion program.

The results are represented by tables and graphs, indicating the mean±SD and the coefficient of variation (CV). The tests of statistical assumptions verification (normality, equality of variances, independence) indicated that cell growth and physicochemical parameters do not comply with these assumptions. So the data was transformed using the growth index model (Formula 1), denominated by some authors as simple growth rate or specific growth rate (SGR) (Molina et al 2001; Travieso et al 2001; Briassoulis et al 2010; Sipaúba-Tavares 2017; Imués-Figueroa et al 2018; Asselbron et al 2015). With data fit to a normal distribution, there was proceeded to the parametric tests (regression and ANOVA) and Tukey's test to compare means; in all cases, and $\alpha=0.05$ was considered.

$$\left(\mathrm{SGR} = \frac{\mathrm{Ln}(\mathrm{D_f}) - \mathrm{Ln}(\mathrm{D_i})}{\mathrm{t}}\right) \qquad (1)$$

Where:
SGR - simple growth rate;
Ln - natural logarithm;
D_f - final density;
D_i - initital density;
t - time (days).

Algal density. The counting method was used, for which the algal density was determined using an Olympus CX 21 brand optical microscope, with a 40X objective, to make the counts on a Neubauer camera, in triplicate, following the methodology reported by Sipáuba-Tavares & Rocha (2003), whose final number is expressed as $N\times10^6$ cells mL^{-1}.

Simple growth rate (SGR). It is a measure of the average increase or decrease of a population in a given period. The model applied by Imués-Figueroa et al (2018) was followed, using Formula 1.

Control variables. In order to guarantee the stability of the water quality variables were periodically controlled in the TPHBRs. The temperature (23.99±0.70°C) and dissolved oxygen (DO=4.30±0.52 mg L^{-1}) were measured three times a day (using a YSI® oximeter, model 550 A for the DO). The pH (7.17±0.65) was measured twice per day with a pH-meter model 8010 VWR Scientific®. The carbon dioxide (0.61±0.40 mg L^{-1}) was measured twice per day following the Standard Method protocol code 272013.

Daily data of internal light intensity (6,696±2,206 lux) was obtained using a DR-meter® lux meter model LXB30B with a sensibility ranging from 0.1 to 200,000 lux. The fluid flow rate (0.08±0.02 L s^{-1}) was evaluated using the K24 Electronic turbinemeter® flowmeter. The fluid velocity (0.16±0.04 L s^{-1}) was calculated using the formula V=Q/A (V-average velocity; Q-flow; A-section area).

The air flow rate (0.74±0.23 L min^{-1}) was recorded at the beginning and at the end of each run, to control the speed of a flow generated from a 3.5 HP blower. The air flow rate was measured by contrasting the variation of the differential pressure gauge Δh (height difference), using alcohol of sg=1.71 kg m^{-3} (specific gravity) as manometric fluid (Mott 2006), with a control anemometer (type Company 4332 Enviro-Meter™). The calibration and standardization equations obtained were: $Q=0.4488\ \Delta h^{0.615}$, for the HTPHBR; $Q=0.5539\ \Delta h^{0.545}$ for the CTPHBR; $Q=0.6145\ \Delta h^{0.522}$, for the CLTPHBR (where Q is measured in L m^{-1} and Δh in cm), for a nozzle meter with the hole diameter of 0.635 cm, which guarantees a constant flow.

Results and Discussion

Chlorella sp. microalgae growth curve. Figure 2 shows the growth curves of *Chlorella* sp. for each type of TPHBR. The cell density values correspond to the averages of four runs, adjusted by moving average, as a way to predict the real behavior of microalgae growth, creating series with averages for periods of two days, given that it is a variable with a second-order effect (Gras 2001; Bannock et al 1999; Daza-Pinzón 2018). Additionally, the fit of the data is identified in a scatter plot.

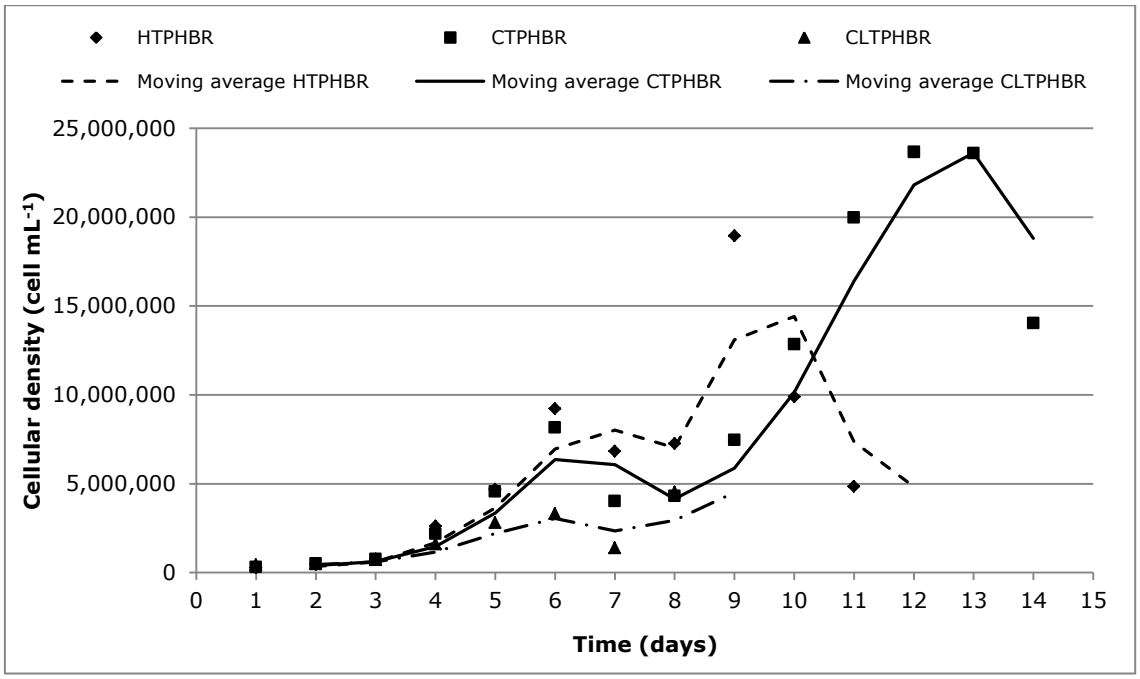

Figure 2. *Chlorella* sp. growth curve in different types of TPHBR with moving average adjustment, based on the average density per sample (cell mL^{-1}).

There was observed that population growth showed a similar behavior during the first four days, corresponding to the adaptation phase, which was longer compared to other sources that report a three days adaptation period. The dynamics behavior in the growth of the strain is associated with an adaptation period since the size of the initial inoculum was in the early logarithmic phase (Polo & Vargas 2019).

This allows intuiting that the type of PHBR strongly influenced cell growth and culture time, with the highest values in CTPHBR (23.666×10^6±887,568 cell mL^{-1}) at 12 days of culture, with a simple growth rate (SGR) of 0.72±0.31% $hour^{-1}$. On the other hand, HTPHBR reached the highest cell density (18.937×10^6±923,592 cell mL^{-1}) at nine days, with a SGR of 3.0±0.18% $hour^{-1}$ and according to the moving average forecast could reach 13.0903×10^6±8.2685×10^6 cell mL^{-1} at 10 days; CLTPHBR obtained its highest cell density at six days of culture (5.038×10^6±939,354 cell mL^{-1}) with a SGR of 1.90±0.24% $hour^{-1}$, which is lower compared to conical and helical TPHBRs, but higher than those found by Polo & Vargas (2019) for the growth of *Chlorella* sp. in a vertical

PHBR system, whose maximum cell density was 3.68×10^6; 3.2×10^6 and 3.26×10^6 cell mL^{-1} in municipal wastewater.

The values found in the present study are higher than those found by Nwoba et al (2016) in the growth of *Scenedesmus* sp., at seven days of culture (34×10^4 cell mL^{-1}) and lower than the density reached in the *Chlorella vulgaris* culture (51×10^6 cell mL^{-1}) after 12 days in TPHBR, reported by these same authors. The results found are also lower to those recorded by Chiu et al (2008) in the culture of *Chlorella* sp. (3.2×10^7 cell mL^{-1}), and similar to those of Robles-Heredia et al (2016) (14.3×10^6 cell mL^{-1}) after five days of culture in annular PHBR.

Chiu et al (2008) observed that the initial behavior of the inoculum cell is an essential factor in growth, since growth is greater (1×10^8 cell mL^{-1}) when cell concentration is higher in eight days of the experiment when he used an inoculum of 8×10^6 cell mL^{-1} of *Chlorella* sp. grown in a semi-continuous PHBR, concluding that growth also depends on the microalgae metabolic and environmental conditions (temperature, dissolved oxygen, pH, CO_2).

From these arguments and the analysis of Figure 2, similarities are identified in the latency phase or establishment, during the first four days, during the cells adaptation to the conditions of the reactors, when the exponential growth phase begins with similar behavior in the HTPHBR and the CTPHBR. The STPHBR does not have this phase as pronounced as in the other reactors. There are two growth transition phases in all systems: around days 6 and 8, in HTPHBR and CTPHBR, a second exponential phase occurs until the start of the stationary phase and between days 11 and 14 a decay occurs, due to the aging phase. In the CLTPHBR, a transition phase is underdeveloped and, later, on a ninth day, the aging period begins, similar to that reported by Plasencia (2012). According to this description, the CTPHBR obtained better behavior, in terms of synchronization of the phases with the best periods.

Chlorella sp. microalgae cell growth prediction with the adjusted model. The theoretical model of the curves responds to a potential equation of the type $Y=aX^{\beta}$, where a is the point of intersection with X and β the slope of the curve, which represent the regression adjustments for cell growth in each TPHBR (Table 1), estimated by an average of the four runs. The value of the coefficient of determination for each case is also indicated as an adjustment measure.

Table 1

Estimated model for the cell growth of the *Chlorella* sp. microalgae in each PHBR and four runs during the cultivation time

Run	*HTPHBR*	*CTPHBR*	*CLTPHBR*
1	Exp[11.8258+1.99799× Ln(days)]	Exp[11.7929+1.63485× Ln(days)]	Exp[12.5441+1.18844× Ln(days)]
2	Exp[11.15+2.09855× Ln(days)]	Exp[11.1158+2.08405× Ln(days)]	Exp[11.8756+1.03706× Ln(days)]
3	Exp[12.1188+2.10188× Ln(days)]	Exp[12.3961+1.80616× Ln(days)]	Exp[12.7831+1.17249× Ln(days)]
4	Exp[12.3497+1.95112× Ln(days)]	Exp[12.3964+2.07713× Ln(days)]	Exp[12.8432+1.30556× Ln(days)]
Average	Exp[12.2322+1.7358× Ln(days)]	Exp[12.1629+1.774× Ln(days)]	Exp[12.6634+1.11604× Ln(days)]
R^2 (%)	85.73	91.88	77.33
r	0.93	0.96	0.88

From the analysis of the estimated regression models, a higher value of the exponent is deduced in runs 3 and 4, which indicates an accelerated growth in a shorter time, with a similar behavior for the three types of TPHBR.

The coefficient of determination indicates the variability of the cell density that is explained by the time variation, with better adjustment in the CTPHBR by having a higher

coefficient of determination (R^2=91.88%), followed by the HTPHBR (R^2=85.73%); finally, the lowest adjustment corresponds to the CLTPHBR (R^2=77.33%). It's probably due to its shorter cultivation period and lower population growth, without the post-exponential phase, but also due to fluid losses due to the number of accessories in the design, causing the cells to settle in the curves of the coil, which affects the ideal conditions for its cultivation and triggers a faster period of senescence (Hernández & Labbe 2014).

The correlation coefficient (r) is higher in the CTPHBR (0.96), followed by the HTPHBR (0.93), cell density and cell population lifespan having a robust linear relationship in these two systems, and lower in the CLTPHBR (0.88), expressing a significant positive linear relationship between time and cell density.

In Figure 3, it is possible to observe the adjusted and normalized curves generated from the estimated models (Table 1) for the average of the runs.

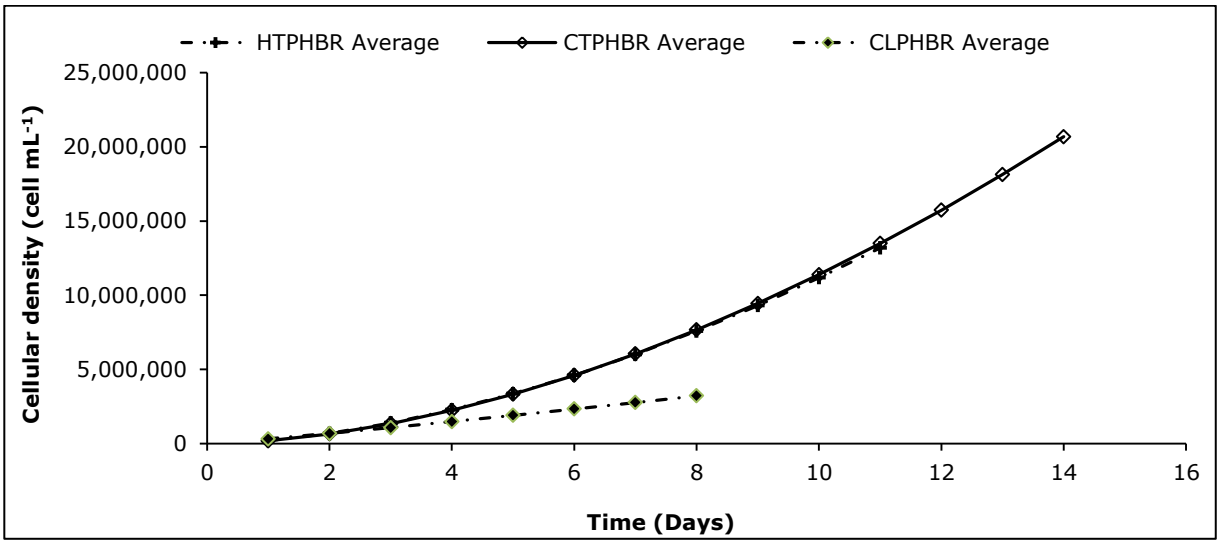

Figure 3. *Chlorella* sp. growth curves adjusted and normalized in the different photobioreactors based on the estimated model.

Coincidence in latency phase curves was observed during the first three days due to adaptation of cells for the TPHFB conditions. This coincidence continued in the exponential growth phase between helical and conical, while serpentine described a curve with a smaller slope. Accordingly, it was estimated that the highest cell growth in the CTPHBR can reach 20.678×10^6 cell mL^{-1} at 14 days of culture and the HTPHBR can reach 13.183×10^6 cell mL^{-1} at 11 days; finally, the CLTPHBR can achieve its maximum cell growth (3.217×10^6 cell mL^{-1}) at eight days of culture. Similar prediction curves indicate matching predictions for HTPHBR and CTPHBR in cellular growth and an extended observation period for the CTPHBR, more biomass can be expected in longer growing time.

Comparison of intermediate growth. Due to the differences in the cultivation period for the TPHBRs, a cut was made at six days, as a comparison time, at which time the beginning of the exponential growth phase can be seen, according to the growth curves (Table 2). At this point, an average density of $6.892\times10^6\pm5.282414\times10^5$ cells mL^{-1} was obtained, and SGR of 46.33±16.34% daily.

Table 2

Standardization on day six as a time of comparison of *Chlorella* sp. culture in the different photobioreactors

	Cell density (cell mL)				*SGR*
General	*Average (cell mL^{-1})*	*Days*	*SD (cell mL^{-1})*	*CV (cel mL^{-1})*	*Average (% day^{-1})*
average	6.892×10^6	6	5.282×10^6	76.64	46.33±16.34
HTPHBR	9.216×10^6	6	5.638×10^6	61.18	58.19±7.71 A
CTPHBR	8.158×10^6	6	6.412×10^6	78.60	53.50±5.33 A
CLTPHBR	3.304×10^6	6	487.074	14.74	27.90±12.32 B

SD-standard deviation; CV-coefficient of variation. Different letters in the same column show significant differences ($p<0.05$).

The ANOVA, at this point of comparison, showed no significant differences ($p>0.05$) between runs. A one-way analysis of variance was carried out, in a completely randomized design with sub-sampling, to compare the treatments (TPHBRs). No significant differences ($p>0.05$) were encountered when the population density was compared between the HTPHBR ($9.216\times10^6\pm5.638\times10^5$ cell mL^{-1}) and the CTPHBR ($8.158\times10^6\pm6.412\times10^5$ cell mL^{-1}). However, differences were significant ($p<0.05$) when the means are compared (Tukey HSD) with the CLTPHBR ($3.304\times10^6\pm487{,}074$ cell mL^{-1}); the variability (CV=14.74%) was significantly lower than for the two other reactors, probably due to a lower growth, which makes the cell population more stable.

At six days, as a comparison time in the culture of *Chlorella* sp., the HTPHBR has the highest SGR (58.19±7.71% day^{-1}), followed by the CTPHBR (53.50±5.33%), and the CLTPHBR with a lower SGR (27.90±12.32% day^{-1}). These values are higher than those found by Astocondor et al (2017), with 42.2±2.6%, in their study on population growth and productivity of the native microalgae *Chlorella peruviana*. This behavior is due to the design of the CLTPHBR, which determines a lower fluid flow rate (0.05±0.02 L s^{-1}) and speed (0.10±0.04 m s^{-1}) than in the other reactors. The physiology of the microalgae is also affected with the cell growth, due to the adhesion of the cells to the surface of the PHBR, which reduces the availability of light in the culture. Therefore, the length of the the CLTPHBR determines an accumulation of oxygen that can be harmful to the cells, by generating a photooxidation phenomena. Besides, due to the consumption of CO_2, the concentration at the ends of the tube is lower, which causes a decrease in the photosynthetic activity and productivity of the crop (González-Céspedes 2016).

Population growth and SGR in the culture period. Table 3 shows the average values of cell density and SGR of *Chlorella* sp.

Table 3

Average cell density (cell mL^{-1}) and SGR of *Chlorella* sp. in three types HTPHBR, CTPHBR, CLTPHBR, during four runs

		Cell density (cell mL^{-1})		*SGR*	
	Days	*Average (cell mL^{-1})*	*SD (cell mL^{-1})*	*Average (% hour^{-1})*	*SD (% hour^{-1})*
Run	8	3.934×10^6	5.260×10^6	1.82	2.21
1	8	2.458×10^6	2.125×10^6	1.569 A	2.24
2	11	5.096×10^6	7.298×10^6	1.250 A	2.37
3	6	3.354×10^6	4.306×10^6	2.488 B	2.03
4	6	3.745×10^6	4.277×10^6	2.534 B	1.93
PHBR	8	3.934×10^6	5.260×10^6	1.82	2.21
HTPHBR	8	4.154×10^6	4.783×10^6	2.390 A	2.35
CTPHBR	9	5.147×10^6	6.864×10^6	2.142 A	1.80
CLTPHBR	7	1.688×10^6	1.495×10^6	1.349 B	2.51

SD-standard deviation. Different letters in the same column show significant differences ($p<0.05$)

The average SGR run^{-1} and by TPHBR was 1.82±2.21% $hour^{-1}$, with significant differences ($p<0.05$) between runs, in double track ANOVA, in a randomized complete block design to compare the TPHBRs. There were no significant differences ($p>0.05$) when comparing the SGR $hour^{-1}$ in the HTPHBR (2.39±2.35%) and in CTPHBR (2.142±1.80%), but SGR $hour^{-1}$ was significantly different ($p<0.05$) when the means were compared with the CLTPHBR (1.349±2.51%). These rates were higher than those reported by Colorado et al (2013) in their research, with a hourly growth rate of 0.71% in a crop of *Chlorella* sp., who observe that elevated growth rates in microalgae are mainly due to the positive stimulation of physical-chemical conditions and water quality in PHBR.

Determination of chlorophyll-a. The estimated averages of chlorophyll-a did not show significant differences ($p>0.05$) when HTPHBR (1.88±0.26 mg m^{-3}) and CTPHBR (1.88±0.20 mg m^{-3}) were compared. However, the CLTPHBR showed significant differences ($p<0.05$) respect to the other systems, with a lower average (1.42±0.27 mg m^{-3}) and low variability (CV<20%) (Table 4). That is possible because they obtained a higher cell density and the CLTPHBR did not reach its maximum cell density at the beginning of the exponential phase, showing a green color with light shades of light green, presence of foam and bad smell in the crop, due to the lack of nutrients (Valdez et al 2018).

Table 4

Concentration average of Chlorophyll-a (mg m^{-3}) of *Chlorella* sp., in three types of TPHBR

	Chlorophyll-a (mg m^{-3})	*SD (mg m^{-3})*	*CV (%)*
General average	1.726	0.25	14.47
HTPHBR	1.88 A	0.26	14.02
CTPHBR	1.88 A	0.20	10.80
CLTPHBR	1.42 B	0.27	19.18

Where SD is standard deviation and CV is coefficient of variation. Different letters in the same column show significant differences ($p<0.05$).

The loss of green color as the microalgae adapt to heterotrophic conditions is due to the biodegradation of chlorophyll, caused by the lack of light and nitrogen, necessary for photosynthesis (Rodríguez-Castillo et al 2016). The ability of algae to carry out photosynthesis makes their nutritional requirements simple: if they have access to light, they can grow in entirely inorganic media. The concentration of pigments varies between species and depends on external (light, temperature, availability of nutrients) and internal (algal physiology) (Brennan & Owende 2017) parameters.

Similar behavior was observed in the study carried out by Rivera et al (2005), who compare the estimation of chlorophyll-a by spectrophotometric and fluorometric methods. They conclude that the microalgae pigmentation is related to the availability of nutrients and effect of other external parameters. Additionally, it has been recorded by various authors that there is no precise relationship between pigment concentrations and biomass (Calderón-Delgado et al 2019).

The values found in this investigation are higher than those reported by Rivera et al (2005), in terms of cell density (2.240×10^{6}±0.070×10^{6} cell mL^{-1}) and chlorophyll-a concentration (0.939±0.023 mg m^{-3}) for the *Porphyridium cruentum* microalgae, but lower than those found by Romero-Maza et al (2017) (11.08±0.006 µg mL^{-1}) in a helical PHBR, while cultivating *Arthrospira maxima*. Otero et al (2013) evidenced chlorophyll-a concentration values (1.05±0.14 µg L^{-1} and 0.74±0.15 µg L^{-1}) comparable with the results of the current study, in *Chlorella vulgaris* cultures.

Conclusions. Culture time, concentration of inoculum, and PHBR type affected the cell growth of *Chlorella* sp. microalgae, with higher cell concentrations at the end, when periods were extended, but with a lower growth per unit of time. The curve described two growth transition phases in all systems, marked on days 6 (HTPHBR) and 8 (CTPHBR),

with a new momentum in the exponential phase, until the start of the stationary phase, before the start of the senescence phase decline, on days 11 and 14 respectively.

Exponential regression models indicated a similar behavior in the three types of PHBR, with a better adjustment in the CTPHBR (R^2=91.88%), the HTPHBR (R^2=85.73%) and lower in the CLTPHBR (R^2=77.33%). CLTPHBR curve design missing a post-exponential phase was possibly determined by a shorter cultivation period, and by a lower population growth. The latter was due to fluid losses and to the number of design accessories, causing the cells to settle in certain places, affecting the growing conditions and precipitating the period of senescence.

The coincidence in the latency phase curves during the first three days was due to an adaptation of the PHBR conditions cells, without significant differences, along the entire culture period, between HTPBR and CTPHBR, while the CLTPHBR described a curve with a smaller slope compared to previous ones, with a density that can reach 20.678×10^6 cell mL^{-1} (CTPHBR) at 14 days of culture, 13.183×10^6 cell mL^{-1} (HTPHBR) at 11 days and 3.217×10^6 cell mL^{-1} (CLTPHBR) after eight days of cultivation.

Prediction models were adjusted in periods of two days, due to the influence of environmental conditions that affect the growth of *Chlorella* sp., reaching the highest cell density at 12 (CTPHBR), 9 (HTPHBR) and 6 (CLTPHBR) days, with adjustments in the exponential models of daily periods that allow estimates of cell growth with prolongation up to 14, 11 and 8 days.

There were not significant differences between helical and conical reactors, the CTPHBR being the first to reach its highest production in less time (18.937×10^6±923,592 cell mL^{-1} in 9 days) and the highest SGR (3.0±0.18% $hour^{-1}$), compared to the HTPHBR that requires more time (23.666×10^6±887,568 cell mL^{-1} at 12 days of culture) and a lower SGR (0.72±0.31% $hour^{-1}$). At 6 days of cultivation, when the exponential phase begun, the differentiation in the population growth of HTPHBR and CTPHBR started, in contrast to the CLTPHBR, which showed the lowest growth rates.

It is necessary to continue with investigations on the systems, establishing different parameters and other species of microalgae, independent of the area where the PHBRs are operated, in order to guarantee the stability of environmental conditions in the laboratory.

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Authors:
Yeni del Socorro Matabanchoy-Mesias, University of Nariño, Faculty of Animal Sciences, Program of Aquaculture Production Engineering, 520001 Pasto, Colombia, e-mail: jennymar401@gmail.com
Yésica Alexandra Rodríguez-Caicedo, University of Nariño, Faculty of Animal Sciences, Program of Aquaculture Production Engineering, 520001 Pasto, Colombia, e-mail: caicedoyesica25@gmail.com
Marco Antonio Imués-Figueroa, University of Nariño, Faculty of Livestock Sciences, Department of Hydrobiological Resources, 520001 Pasto, Colombia, e-mail: marcoi@udenar.edu.co

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