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Research Article

Optimization of *Chaetoceros gracilis* Microalgae Production for Fish Feeding Using an Airlift Photobioreactor

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Abstract: An experimental procedure was carried out to maximize *Chaetoceros gracilis* growth. *Chaetoceros gracilis*, marine microalgae, is considered for feeding fisheries with no GMO (Genetically Modified Organisms) to avoid human health hazards. Furthermore, following United Nations Resolution on water, the microalgae is grown in photobioreactors due to its low water usage. To maximize the microalgae growth, an experimental design was carried out to analyze the effects of Light Intensity, CO_2 supply per day, Sparger type, Photoperiod and Inlet airflow, pH and water temperature were monitored but not controlled. It was found that Light intensity and CO_2 supply per day have statistical significance. Out of three possible scenarios, 1700 lux and 80 gr/day of CO_2, leads to a cell density at day three of 310×10^4 cel/mL which represents 20% more of the density attained in day two under bag (standard) growing conditions. It was also found that water Ph has also a strong effect over cell density.

Keywords: Airlift, *Chaetoceros gracilis*, food security, optimization, photobioreactor

INTRODUCTION

Food security is one of United Nation´s Sustainable Development Goals, SDG, (United Nations, 2012), Food security assessment still shows a big difference across regions and this confirms that efforts are required to enable access to underserved communities (FAO, 2016). Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms, that despite severe conditions, they can grow rapidly, mainly because of their unicellular or simple multicellular structure (Mata *et al*., 2010). They can be found in any existing earth ecosystems, aquatic and terrestrial as well. It is estimated that there are more than 50.000 species, but only around 30.000 have been studied and analyzed (Cheng and Ogden, 2011). Many authors describe the applications of microalgae for CO_2 removal from industrial flue gases by bio-fixation reducing the GHG emissions (Ma and Hemmers, 2011; Wang *et al*., 2008, Zhou *et al*., 2017), biofuels production (Hossain *et al*., 2008; Meng *et al*., 2009; Gouveia and Oliveira, 2009; Kumar *et al*., 2016), wastewater treatment (phycoremediation) by using contaminated water as nutrient media for algae growth (Olguín *et al*., 2003; Muñoz *et al*., 2009; Laliberté *et al*., 1997; Hodaifa *et al*., 2008; Rawat *et al*., 2016), production of chemical compounds, i.e., cosmetics, pharmaceuticals, nutrition and food additives (Borowitzka, 1999; Pulz and Gross, 2004; Carlos *et al*., 2011; Suganya *et al*., 2016) and live food in aquaculture (De Pauw *et al*., 1984; Richmond, 2000; Leal *et al*., 2016). Microalgae have close interactions with their environments; consequently, surrounding parameters such as temperature, light, culture mixing, nutrient concentrations and pH, must be at optimum levels (Oncel and Vardar Sukan, 2008; Le *et al*., 2012). Light in an important parameter for microalgae growth rate and this rate increases when light intensity increases up to an optimal value, close to a saturation level. Further increase creates photoinhibition and the growth stops. Mixing is necessary to prevent cell sedimentation and clustering, as well as thermal stratification. It is also required for nutrients distribution in addition to break down diffusion gradients at the cell surface, to remove generated oxygen and to guarantee that cells experience light/dark alternating periods (Richmond, 2000). A good mixing system will improve gas exchange while reducing the effects of photoinhibition at the illumination surface and biomass loss in the dark zone (Ogbonna and Tanaka, 2000). The flow regime of the culture medium, mixing characteristics, average irradiance and light regimen at which the cells are exposed, regulate the productivity of the culture. The mixing device and intensity, is dictated by the

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characteristics of the organism to be cultivated. Vertical column PBR seems to be a good option for microalgae cultivation when compared to other common configurations (Roncallo *et al*., 2013; Pires *et al*., 2017). These reactors can have different configurations such as internal-loops, external-loops or divided column airlift systems and bubble columns (Borowitzka, 1999; Posten, 2009; Travieso *et al*., 2001; Ugwu *et al*., 2008; Pulz, 2001). Aeration rates and bubble diameters depend on the spargers, which provide good mixing and good momentum transfer in the reactor. Similarly, different light regimes may be maintained inside the photobioreactor, especially in the downcomer and riser sections, which can create photo inhibition in the culture. The time a cell spends in light/dark zones may be related to the rate of circulation in the downcomer and the riser. Various microalgae species have been tested in different vertical columns PBRs by different researchers with variable degrees of success (Richmond, 2000; Mirón *et al*., 2002; Degen *et al*., 2001; Barbosa *et al*., 2003; Molina Grima *et al*., 2003). Nevertheless, the airlift PBR has shown to be the best alternative for microalgae cultivation (Oncel and Vardar Sukan, 2008; Roncallo *et al*., 2013), furthermore, it requires minimum water and follows United Nations recommendations on drinking water usage (United Nations, 2011). In this study, *Chaetoceros gracilis* production is optimized for animal feeding (Ohs *et al*., 2010; Vu *et al*., 2016) in terms of cell density, using an experimental design method where light intensity, CO_2 supply, sparger type, photoperiod and inlet air flow, were varied.

MATERIALS AND METHODS

Microalga and culture media: *Chaetoceros gracilis* was obtained at CENICACUA microalgae laboratory (Colombian Research Center for Aquaculture). The culture media was a modified Conway, sterilized at 120°C for 15 min.

Description of the photobioreactor: A schematic diagram of the PBR used for the experiments is presented in Fig. 1.

The experiments were carried out using an airlift photobioreactor (PBR). This device was installed in a 0.9 m×0.9 m×1.2 m box. It was made of acrylic with a thickness of 4 mm. The working volume for the PBR is 32 L, with 762 mm height and 250 mm internal diameter. The draft tube (1) of the airlift is 696 mm high, 150 mm internal diameter and has four windows located near the top of the tube to allow water circulation. The sample collection tube is located at the top lid is identified by (2). Illumination intensity is provided by eight 14 W daylight fluorescents tubes (Excelite EXT5, 110-130 V and 50-60 Hz), identified by (3). The device used to fix the porous sparger (4) was located at the bottom of the PBR. The spargers

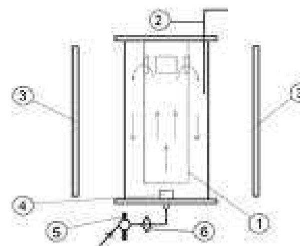


Fig. 1: Diagram of the airlift PBR

were used for generating 3 mm (Type I) and 10 mm (Type II) bubble diameters. The reactor was filled with 32 L of water. Filtered air was supplied by a compressor (5) at a defined flow rate ranging between 1-5 L/min. The airflow was measured with a Dwyer flow meter, ranging between 1-5 L/min (6).

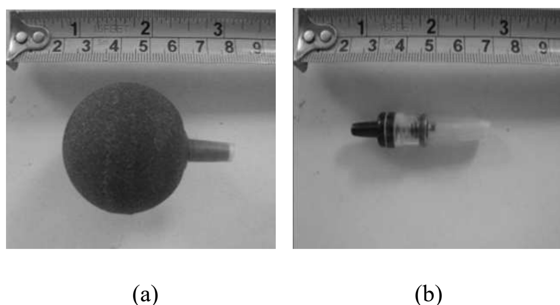
Culture process: The initial cultures for the experiments were obtained according to the following procedure. The media was sterilized via UV lights and alcohol at 70%. The nutrients, strains and inoculants were manipulated after sterilization using a flame. The water used in the experiments was obtained from a nearby ocean shore and was filtered using 0.22 μm filters. The water was exposed to UV lights and sterilized. For the production of inoculants, one of the dilutions was replicated. Several test-tubes were arranged with 9 mL of water along with nutrients. After 7 days, these cultures were transferred as inoculants to 125 mL glass containers. After 7 days, cultures were transferred to 3 L containers. The service area for the cultures was sterilized with alcohol (70%) and sodium hypochlorite (12%). The water used for the 3 L volume cultures was filtered using 1 μm filters, exposed to UV lights and chlorinated water (12 ppm). Before introducing the culture, the PBRs were sterilized using a multi-step procedure. Subsequent to a thorough washing of the parts for several times to remove organic and inorganic residues, PBRs were rinsed with sterile distilled water and sterile sodium hypochlorite solution. After this, the system was emptied and left to dry. Finally, the PBRs were filled with treated seawater to begin the culture.

Cell counting and pH determination: Daily cell counting was done daily in a Neubauer chamber according to Andersen (2005). The pH level was measured daily with a pH meter (Hanna HI 98127).

Experimental design: An experimental design methodology based on orthogonal design was used to optimize the *Chaetoceros gracilis* production in terms of cell density. The first stage of experimentation used a screening experiment to identify significant factors. The factors under consideration were (A) light intensity, (B) CO_2 supply per day, (C) type of sparger, (D) length of the photoperiod and (E) inlet air flow. The definition and ranges for these factors are listed on Table 1.

Table 1: Experimental factors and range

Id	Factor	Experimental range
A	Light intensity	680-2720 lux
B	CO_2 supply per day	30-100 g
C	Sparger	Type I-Type II
D	Photoperiod	18-24 h
E	Inlet air flow	1-5 L/min



(a) (b)

Fig. 2: Sparger Type I (a, 3 mm bubbles) and Type II (b, 10 mm bubbles)

Furthermore, some potential experimental factors were kept constant: the culture media, the water salinity, the temperature (24±1°C) and the total culture volume in the PBR (32 L). The light intensity was changed by varying the number of lights on (340 lux per light). Two types of spargers were used (Factor C). Sparger type I (Fig. 2a) produces bubbles with a 3 mm diameter and Sparger type II (Fig. 2b) produces bubbles with an average diameter of 10 mm.

Two response (output) variables were measured (and later analyzed) during three consecutive days of experimentation for each experimental condition: Cellular density and pH. The three day period was determined because in pilot runs, the airlift PBR culture of *Chaetoceros gracilis* peaked on day 3, then the culture declined. Such decline may be associated with nutrient deficiency, especially nitrogen and increased light limitation to support growth for increased cell density (Anandarajah *et al*., 2012). The experimental design used was a 2_V^(5-1) fractional factorial, enhanced with 4 center points. It was used to determine independent and interacting factor effects on the response variables. The design required 20 runs (16 from the fractional factorial and 4 center points). The randomized experimentation sequence and calculations were carried out using Statgraphics ® Centurion XV, a commercial statistical package. Once the screening stage of experimentation was completed, a full factorial (3K) was run for the significant factors in order to better model the curvature of the response surface and carry out the optimization of cell density. The initial cell density of the culture for each experimental run was kept constant at 20×10^4cel/mL.

RESULTS AND DISCUSSION

Screening experiment: The first stage consisted on a screening experiment with 5 factors and two response variables: cell density and pH. Since each variable was measured during three days, six statistical analyses were performed. Regarding cell density, the most relevant analysis was design factors vs cell density at day 3. Figure 3 shows the half-normal probability plot for the factors and interaction effects, where it can be observed that factors A (light intensity) and B (CO_2 supply) and their interaction appear to be significant.

To confirm the finding, an Analysis of Variance (ANOVA) is performed for the selected effects, as shown in Table 2. Because of the curvature, it is necessary to explore the operational region with more than two levels per factor. For pH, the normal probability plots for day 3 showed that the main factor did not have any significant effect over pH. The ANOVA analysis confirmed this, however, to better understand potential relationships, a matrix of Pearson correlation coefficients between cell density and pH was built leading to Table 3. It can be observed the strong level of correlation between pH on day t and the cell density on day t+1, opening the door for future research addressing pH as a design factor.

Optimization: The significant curvature effect shows the need for more than two levels. Hence, for

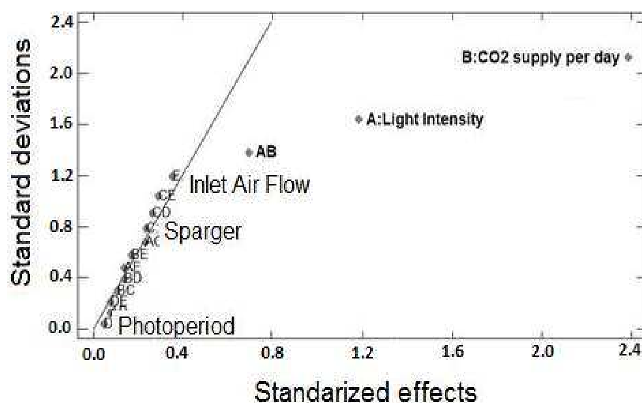


Fig. 3: Half normal probability plot for cell density at day 3 (x104)

Table 2: ANOVA for the 25-1 (nC = 4) design

Source of variation	SS	dof	MS	Fo	p-value
A-Light intensity	9264.1	1	9264.1	40.8	<0.0001
B-CO_2 supply per day	37539.1	1	37539.1	165.3	<0.0001
AB	3164.1	1	3164.1	13.9	<0.0020
Curvature	26100.3	1	26100.3	114.9	<0.0001
Residual	3406.3	15	227.1		
Total	79473.8	19			

Table 3: Response variables correlations

	Cell density		
pH	Day 1	Day 2	Day 3
Day 1	0.66	0.84	0.82
Day 2	0.54	0.76	0.91
Day 3	0.07	0.39	0.68

Table 4: Results from the 32 full factorial design. Data from the experiment

Run	Light intensity (lux)	CO_2 supply per day (g CO_2/day)	Cell density at day 3 (cel/mL)
1	1700	65	300×10^4
2	1700	65	285
3	680	100	205
4	2720	30	160
5	680	30	135
6	1700	100	290
7	1700	65	290
8	2720	100	275
9	1700	65	305
10	2720	65	280
11	1700	65	300
12	1700	30	205
13	680	65	195

Table 5: Results from the 32 full factorial design ANOVA for the 32 full factorial design

Source of variation	SS	dof	MS	Fo	p-value
Model	41066.4	5	8213.3	45.6	<0.0001
A-Light-intensity	5400.0	1	5400.0	30.0	< 0.0009
B-CO_2 supply per day	3612.5	1	3612.5	20.7	<0.0029
A^2	8725.5	1	8725.5	48.5	0.0002
B^2	5896.9	1	5896.9	32.8	<0.0007
A^2B	18.8	1	18.8	0.1	0.7564
Residual	1260.6	7	180.1		
Lack of fit	990.6	3	330.2	4.9	0.0796
Pure error	270.0	4	67.5		
Total	42326.9	12			

optimization, the experimental regression model requires more data, which was obtained from further experimentation using only two significant factors. This is presented in Table 4.

This data set includes missing values from the initial 2^K fractional factorial design to complete a full 32 factorial experiment. The variance analysis for the model can be observed on Table 5. From the above analysis we validate the use of quadratic terms, supported by the fact that the lack of fit is not significant. Out of different alternatives, the best regression model is presented in Eq. (1):

$$Y = -132.06+72.45^{a}+5.85B-6.94^{a2}+ 0.04B^2+0.01^{a2} B \quad (1)$$

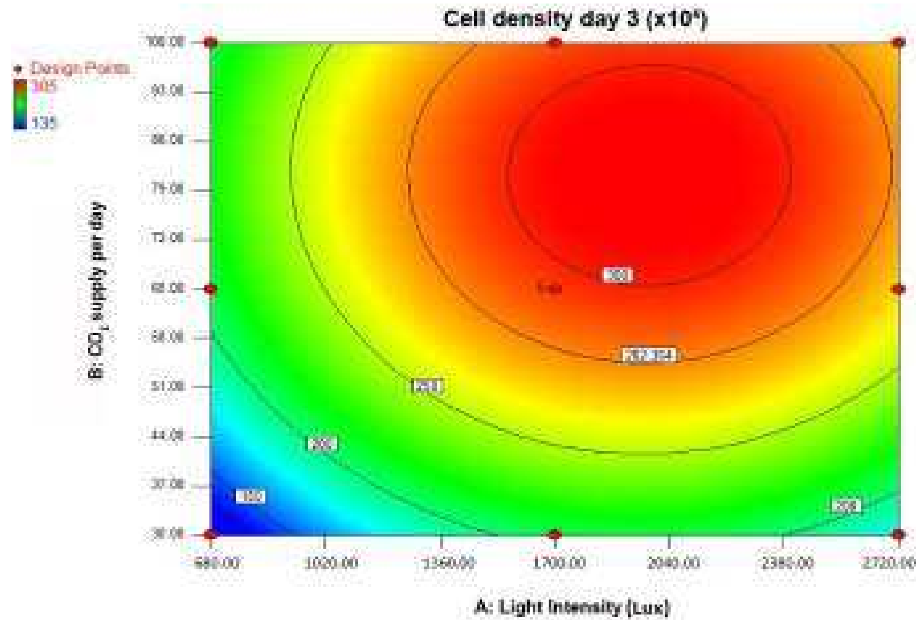
$680 \text{ lux} \leq A \leq 2720 \text{ lux}$

$30 \text{ gr } CO_2/\text{day} \leq B \leq 100 \text{ gr } CO_2/\text{day}$

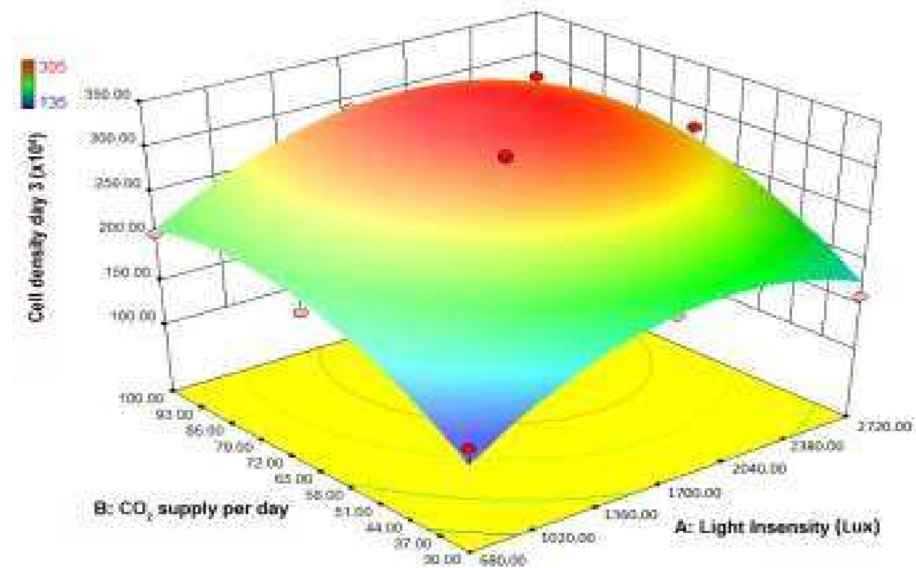
Light control just allowed regulating the number of lights that were on. Hence, factor B (lighting) was not a truly quantitative factor because the experimental set up did not allow for regulation of light intensity in a continuous form. The search for the optimum condition is carried out along vertical lines on the contour plot shown in Fig. 4.

The validity of the model is confirmed by running an ANOVA analysis for the whole regression and it is presented in Table 6.

Design Expert is used in order to obtain the three search scenarios to be run for optimum cell density. The proposed scenarios as well as the estimated cell density at day three are the following Scenario 1 (1700 lux; 79.80 CO_2g/day; 305×10^4 cells/mL), scenario 2 (2040 lux; 83.3 CO_2g/day; 308.9×10^4 cells/mL) and scenario 3 (2380 lux; 81.7 CO_2 g/day; 300.9×10^4 cells/mL). These scenarios indicate the recommended (optimum) CO_2 supply rate for every light intensity level between



(a)



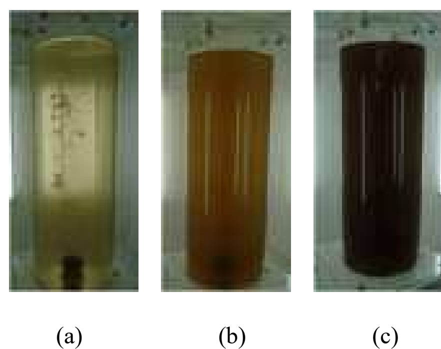
(b)

Fig. 4: Cell density at day 3 (x10^4); (a): Contour plot; (b): Response surface

Table 6: ANOVA for the selected model

Source of variation	SS	dof	MS	Fo	p-value
Regression	41572.4	5	8314.5	77.1	<0.0001
Residual	754.5	7	107.8		
Total	42326.9	12			

1700 and 2380 lux and the estimated cell density for each scenario (best cell density expected) at day three. Although scenario 2 shows the best cell density value, it can be argued that scenario 1 achieves a very close value (1.25% less), with 16.6% less light, leading to less energy consumption. To validate the model optimum condition, a final experiment was run following scenario 1 (1700 lux, 80 g of CO_2/day) and using: sparger type II, a photoperiod of 18 h and an air flow of 3 L min/day. Figure 5 shows pictures of the evolution of the PBR during 3 days. The final cell density (at day 3) was 310×10^4celmL/day. This result



(a) (b) (c)

Fig. 5: PBR at day 1 (a), day 2 (b) and day 3 (c); Condition: 1700 lux, 80 g of CO_2/day, sparger Type II, 18 h photoperiod, 3 L/min air flow; (a): 80×10^4cel mL/day; (b): 260×10^4cel mL/day; (c): 310×10^4cel mL/day

indicates a 1.64% deviation from the predicted value obtained from the model for scenario 1.

According to the results shown in Fig. 5, it is noticed that light control and CO_2/day are the main variable controlling culture growth. The values used to obtain the results introduced in Fig. 5, are observed at the top of the response surface presented in Fig. 4.

These results were compared to Pérez *et al*. (2017). These authors present the growth of *Chaetoceros gracilis* from a 80 L bubble photobioreactor, using CO_2 in the air jet. The bublle bioreactor attains a biomass increment of 1, 3 and 7 (mg/L)/d for day 1, 2 and 3 respectively. The results attained in the Air Lift Photobioreactor used in this research, showed biomass increment per day of 18, 54 and 15 (mg/L)/d for day 1, 2 and 3 respectively. It is notices that this kind of devices are well designed to improve cell growth. Furthermore, when compared with the standard values used for Chaetoceros *gracilis* growth in bags, it was observed that at day 2, the Air Lift PBR attained the same cell density when compared to day three in a bag.

CONCLUSION

Out of the five factors considered, two have statistical significance: Light intensity and CO_2 supply per day. Out of three possible scenarios, 1700 lux and 80 CO_2 g/day leads to day three cell density of 310×10^4cel/mL. Sparger type did not result significant. The photoperiod did not impact significantly growth rate, hence a 18:6 light ratio is used to save energy. A strong pH correlation for growth rate was observed. Finally, a regression model was developed to predict cell density of *Chaetoceros gracilis* after three days, as a function of light intensity and CO_2 supply per day.

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CONFLICT OF INTEREST

There is no conflict of interest regarding the manuscript, the results and the publication of the study.

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