Study of the hydrodynamic effect in column PBR on cellular growth, nitrogen removal, lipid productivity and fatty acid profile in *Chlorella vulgaris*.

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Abstract: In this work we analyzed different biochemical parameters such as cell growth, nitrogen removal, lipid productivity and fatty acid profile in *Chlorella vulgaris* by hydrodynamic effect varying the aeration to (0.75, 1.25, 1.75, 2.25) vvm and white light conditions continuous in column photobioreactor; hydrodynamic calculations of the FBR were carried out to determine the shear rate and possible existence of hydrodynamic stress at the proposed aeration conditions; the values reached in the shear rate were reduced (0.0025 to 0.0220) s^{-1}, observing flow of homogeneous type in all the experiments; however, the maximum values of cell growth and specific growth rate (μ) were (5.90x106 cells mL^{-1} and 0.0229 d^{-1}) respectively, as well as the highest N consumption (60%) and the highest productivity of lipids (8.98 $mgL^{-1}d^{-1}$) were reached during the experiment at 0.75 vvm. In relation to the analysis of the fatty acid profile greater presence of polyunsaturated fatty acids (PUFA) was observed in the experiments at 0.75 vvm, 1.75 vvm and 2.25 vvm, however, at 1.25 vvm, higher productivity of saturated fatty acids (SFA) was obtained; with respect to monounsaturated fatty acids (MUFA) the highest concentration was reflected at 0.75 vvm. The components with the highest presence in the fatty acid profile analysis were C12: 0; C20: 5N3; C24: 1; C 22: 0; C22: 2.

Keywords: Aeration; hydrodynamic; photo-bioreactor; lipids productivity; fatty acid profile

Introduction

Population worldwide must solve problems related to energy shortages, due to the reduction in fossil fuel reserves (Harun et al. 2011, Rawat et al. 2011). Hence the importance of exploring new renewable energy sources (Park et al. 2011). Thus, algal biomass could satisfy about 25% of the world's energy needs, also providing other biotechnological products (Rawat et al., 2011). The cultivation of microalgae has attracted a lot of attention, mainly due to its applications in the sequestration of CO_2, production of biofuels, for human consumption and animals, as well as to produce high quality biomolecules. It is estimated that there are **many** algal species that are in the range of 350,000 to 1,000,000, however, around 30,000 species have been studied and analyzed (Richmond 2004). Microalgae are microorganisms that can coexist in various natural habitats. Most of these microorganisms are considered photosynthetic, however some microalgae can reproduce in a mixotrophic or heterotrophic manner (Lee 2004). The general requirements for a successful microalgal culture should include light (photosynthetic and mixotrophic), carbon, macronutrients such as nitrogen, phosphorus, magnesium, and silicates, as well as different micronutrients (depending on the species).

Certain species of microalgae naturally contain a large amount of lipids, these compounds can be increased by variations in different factors such as: intensity and type of light, variations in temperature, salinity, intensity of agitation, etc. (Mata et al., 2010; Widjaja et al., 2009).

Some species of microalgae can store significant amounts of triglycerides or triacylglycerols (TAG), which are the raw material for producing biodiesel. The production of biodiesel from microalgae not only depends on the biomass reached, but also on the oil content contained in each cell.

In particular, the microalgae *Chlorella vulgaris* is often used in biodiesel production studies and other valuable metabolites, due to its ability to adapt under different growing conditions and even to grow using wastewater as culture medium. (Li et al., 2012). Microalgae can be cultivated in open systems such as lagoons or ponds, or closed systems known as photobioreactors (PBR) where there is greater control of different factors that can benefit the production of biomass, lipid production and other high quality products. The PBRs are made of transparent materials and different geometries, they are illuminated internally or externally; They present high productivity, improve photosynthetic efficiency and CO_2 fixation capacity. Its drawback is its high cost, which is why an economic improvement is necessary

in the creation of PBR (Ho et al., 2012). In this type of systems, different aeration rates can be used that can influence the growth of microalgae during the cultivation process; Mixing helps the cells within the PBR have access to the light source and prevents oxygen accumulation in the medium, in the same way it prevents microalgae from adhering to the walls of the photobioreactor or precipitating, which could cause a cell collapse and death (Contreras-Flores et al., 2003).

Proper photobioreactor design is one of the most important tools for the development of phototrophic technology. Some considerations in the design of photobioreactors are: 1) Efficiency in the use of light energy, 2) Ease of scaling, 3) Efficient mixing and 4) Control of side reactions. In addition, a low hydrodynamic stress on photosynthetic cells is recommended; to achieve this situation, it is necessary to take into consideration certain hydrodynamic conditions of importance for the design and performance of column photobioreactors, such as: gas hold up (remaining gas), surface velocities of liquid and gas inside the reactor, in addition to estimating the rate cutting. (Grobbelaar, 2010; Del campo et al., 2007; Piccolo, 2008; Ugwu et al., 2008; Monkonsit et al., 2011; Hincapie, 2010). It is necessary to consider an adequate aeration rate, so that the cells do not sediment and cell death occurs due to the absence of light. Similarly, there is an upper limit on the acceptable level of turbulence, because hydrodynamic forces have a stimulating effect on the physiological processes of the algal cells. The gradual increase in turbulence, in some species of microalgae, favors an increase in the growth rate, since the generation of agitation favors the supply of light and CO_2. However, with greater turbulence, growth decreases dramatically, simultaneously increasing the superficial gas velocity causing possible cellular damage (Fernandez et al., 2012).

The main problem in algae culture is shear stress cell damage. Excess agitation is known to cause turbulence, affecting cell structure, slowing growth and metabolite production. On the contrary, insufficient agitation causes sedimentation and cell death. (Ugwu et al., 2008; Zitelli et al., 2006). The mechanical agitation and the rupture of the bubbles inside the reactor are often the cause of hydrodynamic stress, causing a limitation in the growth and metabolic activity of the algal cells (Grobbelaar, 2010; Del Campo et al., 2007). According to what has been commented, in the present work different biochemical parameters of the microalgae *Chlorella vulgaris* were analyzed by hydrodynamic effect, varying the aeration at (0.75, 1.25, 1.75, 2.25) vvm and conditions of continuous white light in photobioreactor of bubble column.

Materials and Methods

Acclimatization of the strain

The microalgae **were** acquired from the stock of the Center for Scientific Research and Higher Education of Ensenada (CICESE), Mexico. The microalgae **were** kept in the culture medium for its acclimatization for 30 days in 250 mL Erlenmeyer flasks, under continuous external illumination using fluorescent cold white light lamps with light emission of 100 µmol m^{-2} s^{-1}. Transfer routines were performed every day in 250 mL Erlenmeyer flasks. The glassware was disinfected with a 5% sodium hypochlorite solution (Robles-Heredia, 2014).

Preparation of the culture medium

For the preparation of the medium at 90 mg L^{-1} de $N\text{-}NH4^{+}$ (C_{90}), 4 L of fresh medium were prepared, for each liter of water 3 mL of nutrients were added with the following composition: 7 mg NaCl, 4 mg $CaCl_2$, 2 mg $MgSO_4 \cdot 7H_2O$, 15 mg KH_2PO_4, 115.6 mg NH_4Cl, dissolved in 1L of water, in the same way, trace metals and vitamins were added considering the technique for culture medium f / 2 (Guillar and Ryther 1962), the medium prepared it is sterilized in an autoclave at 120 oC and 30 atm; it is allowed to cool to add 4 mL of vitamins for each liter of medium.

Preparation of inoculum

For the inoculum, 500 mL of solution at C_{90} were used, maintaining constant stirring for 5 days in order to obtain a concentration of $1x10^6$ cells mL^{-1} (cel x mL).

Preparation and assembly of the photobioreactors

For the assembly of photobioreactors, PET bottles with a volume of 3 L were used. Additionally, it was necessary to use bottles with a volume of 1L using sterile distilled water to hydrate the air for agitation, the outlet air is bubbled in chlorinated water in order not to contaminate the environment, ¼ inch industrial type hoses were used to inject the air; 2 vertical rotameters with level dial to control air flow; in addition to a compressor of 2.5 Hp of power and 8.5 kg cm^{-2} of pressure. Figure 1 exemplifies the mode of operation of a PBR.

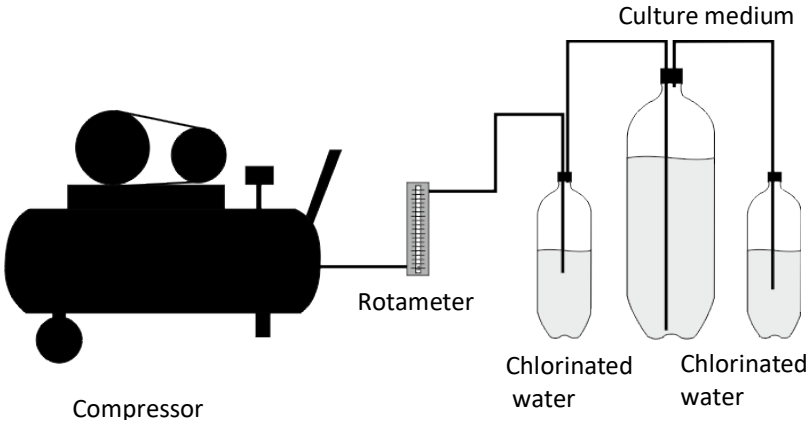

Figure 1. Culture system.

In each PBR, an operating volume of 2 L was used, external illumination, with fluorescent cold white light lamps at 100 µmol m^{-2} s^{-1}. The mixing was carried out by injecting air and the stirring by bubbling consisted in that by gravity the cells descended and by the injection of air they rose, the mixing was continuous during the cultivation process; Figure 2 indicates the variables used to obtain the initial data for the hydrodynamic calculations.

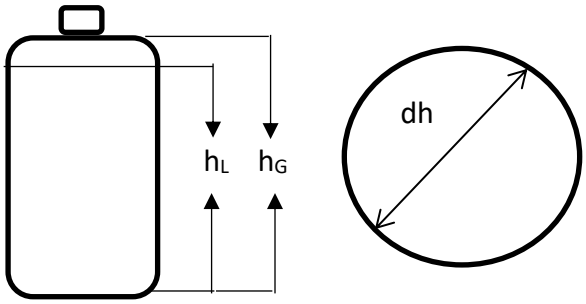

Figure 2. Bubble column equipment dimensions

Where the variables indicated in the figure are the following: h_L is the height of the liquid at rest without air inlet (m); h_G is the height of the column including gas retention (m); dh is the diameter of the bubble column (m) and ρL is the density of the liquid

Aeration conditions

Table 1 indicates the proposed aeration flow values for each experiment, considering a response variable, a configuration, four flows and two repetitions.

Table 1. Experimental design for cultivation in PBR with an operational volume of 2 L

Response variable	Configuration	Aeration rates (vvm)
Lipid productivity	Bubble column	0.75
		1.25
		1.75
		2.25

Procedure to calculate the hydrodynamic parameters in the PBR.

The data indicated in Table 2 were considered to perform the calculations corresponding to the shear rate.

Table 2. Initial data to perform the shear rate calculation process (Y) in the PBR.

vvm	h_G
0.75	0.21
1.25	0.22
1.75	0.23
2.25	0.24

The following data were considered constant for all experiments:

h_L = 0.205 m; do = 0.12 m; Ac = 0.036 m^2; ρL = 998kg / m^3.

It is necessary to calculate the total air flow (Fg) expressed in (Fg) expressed in (m^3s^{-1}) corrected by the absolute pressure at the bottom of the reactor, and with Fa as the supplied air flow in (L min^{-1}), as presented in Equation 1 (Robles-Heredia, 2014).

$$\mathrm{Fg} = \mathrm{Fa}\left(\frac{1}{60}\right)\left(\frac{1}{1000}\right) \quad \textbf{(1)}$$

As presented in Equations 2 and 3, the sectional area of the column (Ac) in (m^2) and the superficial gas velocity (Ug), considered as the gas flow per unit area within the system in (ms^{-1}) are calculated, according to Babcock et al, (2002).

$$\mathrm{Ac} = \mathrm{b\ x\ h} \quad \textbf{(2)}$$

$$\mathrm{Ug} = \frac{\mathrm{Fg}}{\mathrm{Ac}} \quad \textbf{(3)}$$

With the Ug values for bubble column, the shear rate (Y) expressed in (s^{-1}) is calculated, as presented in Equation 4, valid in the range of 0.008 <Ug <0.09 ms^{-1}. (Cerri et al., 2008).

$$\mathrm{Y} = \mathbf{1000}\boldsymbol{Ug}^{\mathbf{0.5}} \quad \textbf{(4)}$$

As presented in Equation 5, the retained gas ε is calculated, this determines the percentage of gas or air retained within the equipment due to the increase in the volume of air when it is injected (Doran, 1995).

$$\boldsymbol{\varepsilon} = \frac{\boldsymbol{hG} - \boldsymbol{hL}}{\boldsymbol{hG}} \quad \textbf{(5)}$$

h_L y h_G were defined above (see Figure 2).

As presented in Equation 6, the surface velocity of the liquid (U_L) is calculated, considered as the flow of liquid per unit area within the system (m s^{-1}), can be calculated for bubble columns with a diameter between 0.1 y 7.5 m and 0 <Ug <0.4 ms^{-1} (Doran, 1995)

$$U_L = 0.9\left(gdhU_g\right)^{0.33} \quad \textbf{(6)}$$

Where g is the acceleration of gravity in (ms^{-2}), dh is the diameter of the column in (m).

As presented in Equation 7, Pneumatic power PG / VL in (Wm^{-3}) is calculated, it is considered as the energy or power generated by the gas or air injected into the equipment to exert the agitation movement of the fluid by expansion inside the photobioreactor. (Doran, 1995):

$$\frac{PG}{VL} = \rho LgUg \quad \textbf{(7)}$$

Where ρL is the density of the liquid in (kg / m^3), g is the gravity in (ms^{-2}).

Cell density

The cell count was performed every 24 h with a 0.1 mm Neubauer Hematocytometer chamber; If necessary, with high cell densities, dilutions of the sample were made in 1:10 mL to facilitate the count, later the total number of cells in the count was multiplied by a factor of 10 according to the dilution taking into account as outside the case, as presented in Equations 8 and 9 (Ruiz-Marín et al., 2010):

$$DC = Cells\ counted\ x\ 10{,}000 \quad \textbf{(8)}$$

$$Diluted\ DC = Cells\ counted\ x\ 10\ x\ 10{,}000 \quad \textbf{(9)}$$

Dry weight biomass

10 mL of the medium were filtered with microalgae using a constant weight filter, the filter with the biomass was placed in an oven for 24 h at 130 °C. After time, the filter was placed in a desiccator for a period of 1 h to cool down, subsequently the weight of the dry sample was obtained by weight difference, considering the volume of sample used. The process was carried out every 24 hours until the end of the process (Robles-Heredia, 2014).

Nitrogen consumption

A sample of 50 mL of culture medium was taken every 24 h, it was filtered and 5 drops of H_2SO_4 were added to fix the nitrogen, later the sample was divided into 2 Erlenmeyer flasks with 25 mL each, 5 mL were added. of borate buffer and 4 drops of 6N NaOH. Boric acid indicator solution was added to 2 flasks, each 20 mL, and 3 drops of Shiro Toshiro indicator were added. Subsequently, the samples were distilled in Buchi micro kjeldahl equipment and 50 mL were collected in the flasks with the boric acid solution and titrated with H_2SO_4 0.02 N until the solution turned from green to purple. The concentration of N-NH_4^+ was determined as presented in Equation 10 (Robles-Heredia, 2014):

$$N - NH_4 = \frac{Volume\ of\ acid\ spent x\ 0.02Nx14}{Sample\ volume} x1000 \quad \textbf{(10)}$$

Harvesting the biomass

At the end of the cultivation time, the remaining volume was centrifuged at 10,000 rpm for 10 min to concentrate the microalgal biomass. The recovered biomass was frozen at –4.0 ° C for its conservation, subsequently it was lyophilized for 3 to 5 days and the lyophilized samples were kept refrigerated at 0 ° C (Ruiz-Marín et al., 2010).

Extraction and productivity of lipids

The determination was carried out by the modified method of Bligh and Dyer (1959) as follows: samples of 0.01 g of lyophilized microalgal biomass were placed in threaded tubes, they were mixed with 4 mL of methanol, 2 mL of chloroform and 0.5 mL of distilled water, the samples were subjected to sonication for 15 minutes to rupture the cell wall, immediately they were wrapped in aluminum foil and left to incubate in refrigeration for 24 H at 4 ºC; After the time, the aluminum foil was removed to sonicate them for 5 min and centrifuge them at 4000 rpm for 10 min, the remaining liquid from the tubes was transferred to new tubes with a screw cap and 4 mL of water was added to wash them. , they were vortexed and centrifuged again, the water in the tubes was removed with a Pasteur pipette and the chloroform was evaporated in a water bath, subsequently 2 mL of a 95% hydrochloric acid-methanol mixture was added and they were introduced to a brand digester. Hach DRB 200 for 1 h at 100 ºC, after the digester time has elapsed, they were wrapped in aluminum foil to be stored in refrigeration for 24 h at 4 ºC. Subsequently, 3 ml of hexane were added and they were vortexed forming a bi-phase in which the lower part was extracted with a Pasteur pipette, 4 mL of water were added to the tubes and they were agitated again with vortex, the water was extracted using a Pasteur pipette. They were wrapped again in aluminum foil and kept refrigerated for 24 h at 4 ° C. After the estimated time passed, these were evaporated with a water bath, in the evaporation process 3 mL of hexane were added before complete evaporation, the samples were transferred to vials and left to stand for 24 h. The lipid content in the sample is translated into a lipid composition (% ww^{-1}) on a dry basis w, and this in turn translates into a lipid productivity P_L (in mg L^{-1} d^{-1}), as presented in Equation 11:

$$\boldsymbol{P_L = \frac{w_2 X_2 - w_1 X_1}{t_2 - t_1}} \quad \textbf{(11)}$$

Where Xi is the mass concentration of dry biomass in the middle at time ti.

Profile of fatty acid methyl esters (FAME)

The analysis of the profile of fatty acid methyl esters (FAME) was carried out using an Agilent Technology 7890 gas chromatograph (GC). The analysis of the fatty acid methyl esters (FAME) profile was performed using an Agilent Technology 7890 gas chromatograph (GC). 1µL of the hexane-lipid solution was injected into the GC equipment with flame ionization detector (FID) and separation column DB-23 (60 µm length, 0.32 mm ID, 0.25 µm thickness). Chromatographic conditions: Detector T: 250 ºC; Injector T: 250 ºC; Oven temperature program: 120 ºC for 5 min, increase the temperature at a rate of 10 ºC min to 180 ºC, hold for 30 min. Increase again at a rate of 10 ºC min to 210 ºC and hold for 21 min. (total 65 min); Carrier gas flow: 15 psi; Split: 1: 100; Carrier gas: I have high purity; Injection volume: 1 µL. The fatty acids present in the samples were identified by: Detailed comparison and analysis of the spectra of known fatty acid methyl esters. Check the retention times of the peaks in the sample with the retention times of a commercial standard of fatty acid methyl esters (standard). For the identification of the FAMES, a mixture of fatty acid methyl esters (% weight) SupelcoTM 37 Component FAME was used. The results of the experimental design were analyzed using a full factorial analysis of variance (ANOVA) (α: 0.05) using the STATISTICA V7 program.

Results and Discussion

Hydrodynamic parameters calculated from bubble column PBR.

The results obtained from the experiments carried out in the PBR are presented below, at the cultivation conditions and proposed aeration rates.

As indicated, it was necessary to consider various data as already established to be able to start the calculations according to the proposed conditions to obtain everything essential in relation to the corresponding experiments.
Table 3 shows the results of the calculations carried out to obtain the hydrodynamic parameters of the bubble column PBR.

Table 3. Bubble column hydrodynamic parameters at the proposed aeration rates.

Equipment	Aeration rate (vvm)	$F_{g\,x}\,10^{-4}$ (m^3s^{-1})	$U_g\,x\,10^{-3}$ (ms^{-1})	U_L (ms^{-1})	PG/VL (Wm^{-3})	Shear rate (s^{-1})
Bubble column	0.75	0.25	1.59	0.050	15.55	0.0025
	1.25	0.41	2.65	0.062	25.91	0.0070
	1.75	0.58	3.71	0.069	36.28	0.0130
	2.25	0.75	4.77	0.075	46.65	0.0220

As stated in the report made by (Morales et al., 2015), according to the values of the hydrodynamic parameters in Table 3, the work regime of the present study was in the range of homogeneous bubbling type flow.
Figure 3 shows the relationship between maximum cell density and shear rate at the proposed aeration rates.

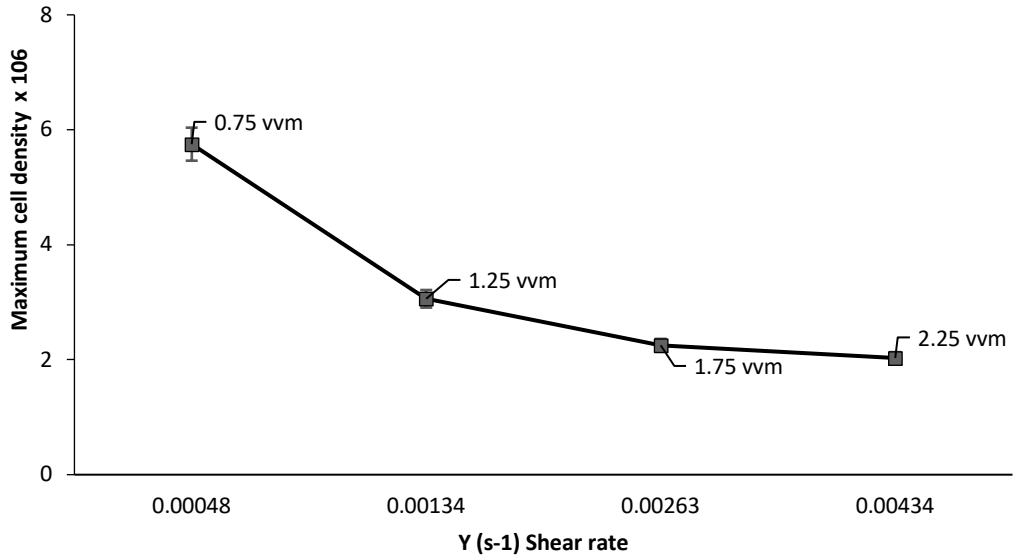

Figure 3. Maximum cell density vs shear rate at indicated aeration rates.

According to Figure 3, it can be seen that as the aeration flow increased, in the same way it was observed that the cutting rate increased, however, the opposite occurred with the cell concentration since as the aeration rate increased, a decrease in the number of cells in each experiment. In relation to the PBR, Valdez-Cruz and Trujillo-Roldan (2006) point out that the main disadvantage of these systems is that the hydrodynamic stress that acts on the cells is variable, due to the irregular changes in the flow in all directions. and in the related time, during each experiment, these are reasons for possible sub-lethal damage in cells due to shear stress (Morales et al., 2015; Trujillo-Roldan and Galindo, 2003; Valdez-Cruz and Trujillo-Roldan, 2006; Robles-Heredia, 2014); Furthermore, another possible cause would be considering the low light intensity in the experiments, causing the cells to not adapt to the conditions of the culture medium, producing these results (Morales et al., 2015).

However, according to what has been observed, it is important to point out that no type of deformation or abnormality was observed in the microalgal cells to verify the existence of sublethal damage due to the aeration rates proposed. Another aspect to note is that the specific growth rate of the experiment at 0.75 vvm compared to the other experiments was higher, despite the expectation that greater cell growth was expected while the aeration flows increased, therefore it can be indicated that it did not happen. What was intended is that with greater aeration flow and moderate light intensity, it would be possible to increase biomass and lipid contents in the microalgae, which would have an effect on higher lipid productivity.

Effect of the aeration rate on cell growth and nitrogen consumption.

Four experiments were performed at proposed aeration rates, under nutrient-rich conditions and continuous white light, to evaluate the effect on different biochemical parameters. Figure 4 shows the cellular growth of the microalgae at the proposed aeration rates (0.75, 1.25, 1.75 and 2.25 vvm), under nitrogen-rich conditions (90 mg L^{-1}).

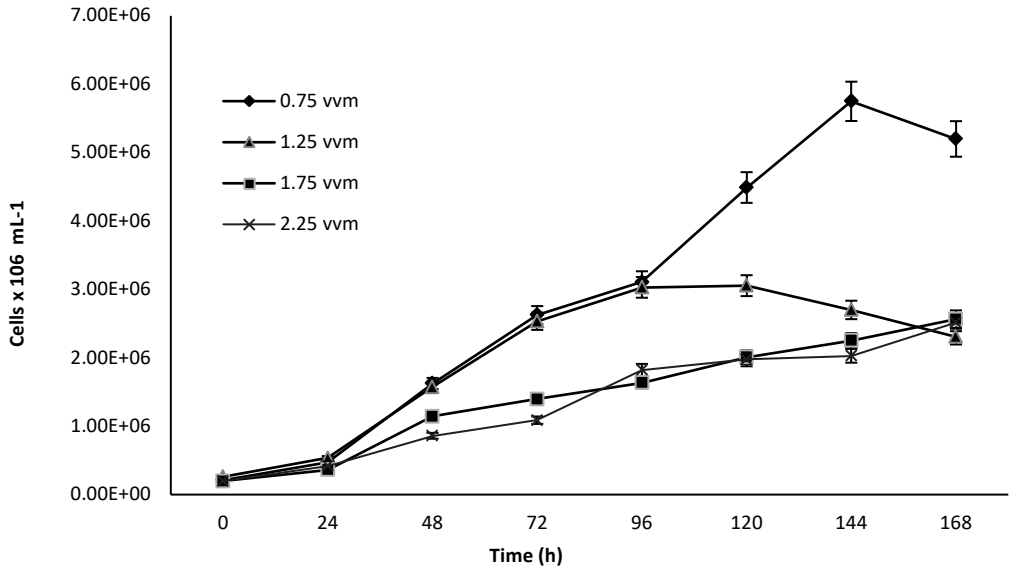

Figure 4. Growth curve of *C. vulgaris* grown in PBR.

Each experiment started at the same cell concentration (1x10^6 cel mL^{-1}), At 24 h an adaptation phase was observed followed by a slight growth in each experiment, however, from 96 h and until 144 h a rebound in growth was observed, presenting an exponential phase during the experiment at 0.75 vvm, with a maximum growth of 5.75x10^6 cel mL^{-1} and immediate decay phase; however, the other experiments (1.25, 1.75 and 2.25 vvm) did not exceed 3.5x10^6 cel mL^{-1}, therefore, it can be inferred that the increase in the aeration rate had a significant impact on algal growth, however, it is important to indicate that the degree of agitation prevailing in each experiment was not causing a negative effect due to shear stress. , since according to the results observed during the development of the culture process, the algal cells did not present any type of deformation, according to what was observed under the microscope, similar aspects were reported by (Sadeghizadeh et al., 2017; Shi et al. al., 2016 and Robles-Heredia 2014).

Table 4 indicates the results of the maximum values reached referring to cell density, specific growth rate (μ) and nitrogen consumption of the experiments carried out.

Table 4. Maximum values of cell density, specific growth rate μ and nitrogen consumption of *C. vulgaris* at the proposed aeration rates.

Equipment	Aeration rates (vvm)	Max cell density* (cell×10^6 mL^{-1})	μ* (d^{-1})	Consumption* N-NH_4^+ (%)
Bubble column	0.75	5.75 ± 2.04[a]	0.0229±1.16[a]	60.00± 2.31[a]
	1.25	3.06 ± 1.18[b]	0.0168±0.90[b]	50.00± 1.91[b]
	1.75	2.25 ± 0.77[b]	0.0162±1.00[b]	49.00± 1.54[b]
	2.25	2.03 ± 0.75[b]	0.0160±0.86[b]	45.00± 1.68[b]

* Different letters in the same column represent significant differences according to the Tukey test (α≥0.05); (± Standard deviation).

According to what is reported in Table 4, it can be pointed out that the strain adapted better to the conditions of the experiment at 0.75 vvm, than to the other experiments, so it can be indicated that the aeration rate influenced its development, obtaining the maximum cell density results of 5.75x10^6 cell mL^{-1}, specific growth rate of 0.0229 d^{-1} and

a nitrogen consumption of 58%. During the experiments at 1.25, 1.75 and 2.25 vvm a nitrogen consumption of less than 50% was observed and its maximum cell density only reached 3×10^6 cell mL^{-1}, in the same way the specific growth rate (μ) does not exceed the 0.0168 d^{-1}; statistical tests of the data obtained under these aeration conditions indicate that there were no significant differences. It can be indicated that the results obtained from growth and removal of nitrogen (N-$NH4^+$), were well below other reported works (Sadeghizadeh et al. 2017; Robles-Heredia et al., 2014), where cell density values greater than 17 $x10^6$ cell mL are reported, specific growth rate greater than 0.18 d^{-1} and consumptions of (N-NH_4^+) up to 90%; the values observed in the present work show what is normally to be assumed, that since there is no increase in cell density, there is no consumption of N-NH_4^+ correspondingly, these considerations were also reported in other works, in a similar way (Robles-Heredia, 2014; Haro and Perales, 2015; Kee-Lam et al., 2016). Given the scenarios of this work, it is possible to mention the possibility that there was some type of disturbance due to the prevailing agitation in each experiment that could even cause some type of photo-inhibition due to the amount of light supplied, affecting cell growth.

Effect of the aeration rate on biomass, lipid content and lipid productivity.

Table 5 reports the maximum values obtained for biomass dry weight, lipid content and lipid productivity at the different proposed aeration rates.

Table 5. Dry weight biomass X, maximum lipid content *wmax* and maximum lipid productivity *PLmax* at the proposed aeration rates.

Equipment	Aeration rates (vvm)	X^* ($g\ L^{-1}$)	w_{max}^* (%ww^{-1})	P_{Lmax}^* ($mg\ L^{-1}\ d^{-1}$)
Bubble column	0.75	0.420±0.28ª	1.03±1.62ª	8.98±3.70ª
	1.25	0.340±0.47^a	0.86±1.61^a	7.98±3.18^a
	1.75	0.298±0.66^b	0.65±3.10^b	6.72±2.48^b
	2.25	0.287±0.84^b	0.62±1.16^b	5.73±1.63^b

*Different letters in the same column represent significant differences according to the Tukey test (α≥0.05); (± Standard deviation).

As observed in Table 5, during the experiment at 0.75 vvm the maximum values of the entire process were obtained, of dry weight biomass with 0.420 g L^{-1} y contenido de lípidos de 1.03%, sin embargo, en relación a la productividad de lípidos, el valor fue similar con la del experimento a 1.25 vvm (7.98 mg L^{-1} d^{-1}).

Studies carried out by Chiu et al., (2008) reported results of 1.2 g L^{-1} biomass at 0.25 vvm and using a light intensity of 300 μmol m^{-2} s^{-1} and Pham et al., (2017) 1.35 g L^{-1} of biomass at 0.3 vvm, with a light intensity of 300 μmol m^{-2} s^{-1}; therefore, it can be indicated that the amount of light supplied has a greater influence on biomass production than aeration. Similarly, in other investigations, they have reported that at higher light intensities, greater than 150 μmol $m^{-2}s^{-1}$, obtained higher amounts of microalgal biomass (Amini-Khoeyi et al., 2012; Hultberg et al., 2014; Jiang et al., 2016; Kim et al., 2015), hence the interest in experimenting with higher aeration flow and with a moderate light intensity it was possible to obtain higher biomass and lipid contents in the microalgae. However, as can be seen, the values reached for maximum lipid content (1.03%) and lipid productivity (8.98 mg L^{-1} d^{-1}) during the experiment at 0.75 vvm, twere reduced compared to other studies where conditions with certain similarities were handled (Mandal and Mallick, 2009; Robles-Heredia 2014).

FAME profile of microalgal lipids.

When the purpose of lipid production in microalgae cultures is the production of biodiesel, not only the lipid productivity is important, but also the fraction of those that can be converted into FAME, that is, the TAG fraction. Also, the types of fatty acids in TAGs are important in terms of the quality of the resulting biodiesel. As can be seen, polyunsaturated fatty acids (PUFA) were the most abundant in the experiments at 0.75 vvm, 1.75 vvm and 2.25 vvm; only in the experiment at 1.25 vvm, a higher productivity of saturated fatty acids (SFA) was obtained.

The statistical test of analysis of variance, showed that there were no significant differences between the SFA and PUFA results, as well as the results of monounsaturated fatty acids (MUFA) in the experiments at 1.25, 1.75 and 2.25 vvm did not show significant differences (p≥0.05), while the MUFA result at 0.75 vvm was slightly higher. Among the main properties of fuels is the cetane number (CN), which is a dimensionless descriptor of the quality of ignition of a fuel and is related to the ignition delay time experienced by a fuel. In addition, the CN depends to a great extent on the nature and structure of a fatty ester, thus it is important to indicate that saturated compounds tend to have a high CN, while the CN decreases with the increase in unsaturation (Knothe, 2010). Regarding the CN and considering the data obtained according to the FAME analysis, the high concentrations of PUFA in the experiments at 0.75 vvm, 1.75 vvm and 2.25 vvm suggest that the resulting biodiesel would be of low quality considering the low presence of saturated fatty acids. Table 6 summarizes the FAME profile found in *C. vulgaris* cultures at the different proposed aeration flows.

Table 6. FAME profile in lipids of C. vulgaris. (Expressed as% in total weight of fatty acids *).

FAME	0.75 vvm	1.25 vvm	1.75 vvm	2.25 vvm
c12:0	23.81 (±0.38)	22.04 (±0.71)	23.73 (±2.02)	20.91 (±5.08)
c13:0	0.96 (±0.27)	0.93 (±0.14)	0.39 (±0.02)	0.84 (±0.69)
c18:0	1.78 (±0.07)	1.48 (±0.038)	1.6 (±0.13)	1.61 (±0.10)
c20:0	0.93 (±0.04)	n.d.	0.21(±0.11)	0.44 (±0.005)
c21:0	n.d.	n.d.	0.27(±0.04)	0.23(±0.07)
c22:0	5.25 (±6.95)	13.36 (±2.25)	8.83 (±8.73)	10.54 (±11.32)
c23:0	0.58 (±0.006)	1.23 (±0.079)	1.22 (±0.061)	1.49 (±0.25)
c17:1	0.85 (±0.11)	0.18 (±0.05)	0.28 (±0.45)	0.49 (±0.073)
c18:1N9T	0.06 (±1.09)	n.d.	n.d.	n.d.
c20:1	1.84 (±0.10)	0.5 (±0.31)	0.82 (±0.074)	1.28 (±0.15)
c22:1N9	2.01 (±0.11)	1.77 (±1.63)	2.6 (±0.20)	2.39 (±0.51)
c24:1	17.22 (±2.71)	7.55 (±1.63)	11.81 (±0.48)	13.1 (±5.41)
c18:2N6T	n.d.	n.d.	n.d.	0.27(±13.06)
c20:2	7.82 (±0.63)	0.46 (±0.053)	7.48 (±8.20)	8.31 (±9.03)
C20:3N3	n.d.	n.d.	0.34(±10.30)	0.74(±7.04)
C20:3N6	0.22 (±0.14)	0.17 (±0.093)	0.24 (±0.13)	0.25 (±0.16)
c20:4N6	3.32 (±0.2)	2.41 (±0.40)	2.98 (±0.13)	2.46 (±0.88)
c20:5N3	21.71 (±2.51)	15.34 (±3.02)	24.14 (±0.81)	24.38 (±8.37)
c22:2	8.35 (±0.31)	9.66 (±1.60)	14.71 (±1.88)	20.46 (±8.49)
c22:6N3	1.78 (±0.25)	1.17 (±0.05)	1.47 (±0.085)	1.51 (±0.34)
SFA	33.34(±8.57)[a]	39.07(±8.67)[a]	36.29(±8.73)[a]	36.10(±7.83)[a]
MUFA	22.00(±7.21)[a]	10.03(±4.97)[b]	15.53(±4.97)[b]	17.27(±5.46)[b]
PUFA	43.23(±7.39)[a]	29.24(±8.75)[b]	51.39(±8.75)[a]	58.43(±9.74)[a]

* Percentages by weight, different letters in the same row indicate significant differences according to the Tukey test (p≥0.05); n.d.=no detected, (± Standard deviation).

Conclusions

The cell growth of each experiment had considerable variations in reference to the aeration rates managed in each one of these; while the aeration flow increased in each experiment, cell growth was negatively affected; It was observed that the highest cell growth rate occurred in the experiment at 0.75 vvm ($5.75x10^6$ cell mL^{-1}) having a nitrogen consumption of 60% of its medium; a higher cell concentration and a higher nitrogen consumption were expected in each test at higher aeration rates added to the PBRs; It is possible that this result occurred due to the fact that while the aeration rates increased in each experiment, the turbulence generated by this, combined with the intensity of light used, could produce a limitation in the photosynthetic activity that interferes in the ability of the cells to reproduce. adapt and consume the $N\text{-}NH_4^+$ from the culture medium.

The experiment at 0.75 vvm obtained the highest results in its lipid productivity (8.98 mg L^{-1}), as well as the highest result in dry biomass productivity (0.420 g L^{-1}); consequently, the amount of biomass obtained at the end of the experiment, specific growth rate and lipid productivity, it is concluded that the experiment at 0.75 vvm in comparison with the other experiments carried out is the most suitable for the productivity of lipids and obtaining biomass. microalgal.

In relation to cell growth, in each experiment at the different aeration rates studied, values were obtained where the initial value of the inoculum was increased, with low results compared to other studies; The experiments carried out showed a homogeneous agitation flow, in addition, it can be indicated that there was no type of hydrodynamic stress damage despite the fact that the calculated cut-off rate increased with greater aeration, this according to the cell growth obtained and the characteristics physical characteristics that the cells presented in the course of the culture process.

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