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OBTAINING FATTY ACIDS OF METHYL ESTERS IN ALGAL BIOMASS AT DIFFERENT AERATION RATES IN COLUMN FBR

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Abstract. Fossil fuels contribute to air pollution, due to the compounds that are released into the atmosphere during combustion; For this reason, they have been proposed to replace them with so-called biofuels, such as biodiesel, which is the mixture of fatty acid methyl esters (FAME) for its acronym in English, which can replace diesel and is obtained from different raw materials, such as biomass. . The variation of cell growth, nitrogen consumption, lipid productivity and biodiesel quality was analyzed depending on the type of FAME of Chlorella vulgaris due to the effect of hydrodynamics in column photobioreactors (FBRC), varying aeration flows at (0.75, 1.25, 1.75, 2.25) vvm and continuous white light; the shear rate was analyzed to verify the probable presence of hydrodynamic stress. The shear rate data were low (26.34 to 45.60) s^{-1} , while the maximum values of cell growth and specific growth rate (μ) were (6.80 x 10^6 cell mL^{-1} and 0.023 d^{-1}), respectively, on the other hand, nitrogen consumption was 65% at 0.75 vvm and lipid productivity was 15.92 mgL-1d-1 at 1.25 vvm. In relation to the FAME, a greater presence of polyunsaturated fatty acids (PUFA) was observed at 0.75 vvm, 1.75 vvm and 2.25 vvm; while at 1.25 vvm, they were saturated (SFA); the highest amount of monounsaturated (MUFA) was at 0.75 vvm. The components with the greatest presence were C12:0; C20:5N3; C24:1; C22:0; C22:2.

Keywords: FAME, aeration, Chlorella vulgaris photobioreactor, hydrodynamics

OBTENCIÓN DE ACIDOS GRASOS DE METIL ESTERES EN BIOMASA ALGAL A DIFERENTES TASAS DE AIREACION EN FBR DE COLUMNA

Resumen. Los combustibles fósiles contribuyen en la contaminación del aire, por los compuestos que se liberan a la atmosfera durante la combustión; por esta razón se han propuesto reemplazarlos por los llamados biocombustibles, como el biodiesel que es la mezcla de esteres metílicos de ácidos grasos (FAME) por sus siglas en inglés, que puede sustituir al diésel y se obtiene de diferentes materias primas, como la biomasa. Se analizó la variación de crecimiento celular, consumo de nitrógeno, productividad de lípidos y calidad del biodiesel en función del tipo de FAME de *Chlorella vulgaris* por efecto de la hidrodinámica en fotobiorreactores de columna (FBRC), variando flujos de aireación a (0.75, 1.25, 1.75, 2.25) vvm y luz blanca continua; además de la tasa de corte para comprobar probable presencia de estrés hidrodinámico. Los datos en tasa de corte fueron bajos (26.34 a 45.60) s^{-1}, mientras que los máximos valores de crecimiento celular y tasa de crecimiento específico (μ) fueron de (6.80 x 10^6 cel mL^{-1} y 0.023 d^{-1}), respectivamente; por otra parte, el consumo de nitrógeno fue de 65% a 0.75 vvm y productividad de lípidos de 15.92 $mgL^{-1}d^{-1}$ a 1.25 vvm. En relación a los FAME, se observó mayor presencia de ácidos grasos poliinsaturados (PUFA) a 0.75 vvm, 1.75 vvm y 2.25 vvm; mientras que a 1.25 vvm, fueron saturados (SFA); la mayor cantidad de monoinsaturados (MUFA) fue a 0.75 vvm. Los componentes con mayor presencia fueron C12:0; C20:5N3; C24:1; C 22:0; C22:2.

Palabras clave: FAME, aireación, Chlorella vulgaris fotobiorreactor, hidrodinámica

Introduction

Reducing the use of fossil fuels is very important to reduce the environmental pollution problem they represent (Castillo et al., 2017). It is important to reduce the use of fossil fuels as they cause emissions of CO_2 , CH_4 , N_2O, nitrogen oxides (NOx), carbon monoxide (CO), non-methane volatile organic compounds (NMVOCs), as well as sulfur dioxide (SO_2), causing damage to the atmosphere, (Chandrasekhar et al., 2015; Kumar et al., 2017; Kumar-Enamala et al., 2018). Hence, the importance of looking for other renewable energy alternatives (Castillo et al., 2017; Anto et al., 2020).

Biofuels obtained from biomass, such as biodiesel, are alternative energy sources that have received more attention for research and use (Qaria et al., 2017; Ashok et al., 2019). Globally, microalgae biomass could satisfy up to 25% of energy needs due to the characteristics of the methyl ester fatty acid compounds they can produce (Castillo et al., 2017). Because of the applications for CO_2 sequestration, producing biofuels, human, and animal food, in addition to the use in the production of high-quality biomolecules, the use of microalgae is to be taken into account (Posten and Feng-Chen, 2016; Alishah et al., 2019; Gomez-Luna et al., 2022).

Despite the considerable number of microalgal species existing in different habitats, approximately 30,000 species have received more attention (Richmond 2004; Agarwal et al., 2018; Chew et al., 2018). Being photosynthetic microorganisms, microalgae can coexist in diverse natural habitats; however, some microalgae can reproduce mixotrophically or heterotrophically (Castellanos et al., 2020). Certain microalgae contain large amounts of lipids, which can be increased by varying different factors such as light intensity and type, temperature variations, salinity, agitation intensity, etc. (Posten and Feng-Chen 2016, Basto-Flores et al., 2022).

Some microalgae can accumulate high amounts of triglycerides (TAG), Kumar et al., (2018), which are the feedstock for producing biodiesel. The amount of biodiesel

using microalgae biomass does not only depend on the amount of biomass, but also on the amount of oil contained per cell (Wu et al., 2017; Chew et al., 2018). Due to its high adaptability to different conditions during cultivation, including wastewater, the microalga *Chlorella vulgaris* has been used for experimentation in the production of biodiesel and other widely used compounds (Li et al., 2012; Zhan et al., 2016).

For the cultivation of microalgae, open systems can be used in the form of ponds or closed systems or photobioreactors (FBR) to better control the cultivation process, amount of lipids, and other products for use in other areas (Kumar et al., 2018). Both types of culture systems present advantages and disadvantages, thus, of the main advantages of open systems is their low cost due to the materials used for their construction. However, the main problem they present is contamination by microorganisms due to their exposure to the environment; while FBRs present other characteristics, internal or external lighting, different configurations, better control of the variables of the type of culture process, and exhibit higher productivities (Ashok et al., 2019); due to these characteristics their main disadvantage is their cost; hence, the need to improve their design. Different aeration rates can be used in FBRs that can influence the growth of microalgae during the cultivation process, mixing helps the cells to have access to the light source and avoids oxygen accumulation in the medium, preventing microalgae from adhering to the walls or precipitating. (Deconinck et al., 2018; De jesus et al., 2019). Some considerations in FBR design are efficiency to harness light energy, easy scale-up, efficient mixing, and better control of side reactions, suggested under cellular hydrodynamic stress.

Among the hydrodynamic conditions of importance for a good performance of column FBRs are gas hold up (remaining gas), liquid and gas surface velocities inside the reactor, in addition to estimating the shear rate (Beal et al., 2015; Gonzalez-Lazo et al., 2019). An adequate aeration rate is necessary, with the idea of avoiding cell sedimentation and cell death due to the absence of light. Similarly, there is an upper limit to the acceptable level of turbulence since hydrodynamic forces have a stimulating effect on the physiological processes of algal cells. A progressive increase in turbulence, in some microalgae favors an increase in growth rate as agitation favors the supply of light and CO_2. However, with high levels of turbulence, growth is decreased, in addition to simultaneously increasing the surface gas velocity causing possible cell damage (Trivedi et al., 2015; Gonzalez-Lazo et al., 2019). Shear stress is one of the main problems in microalgae cultivation due to cell damage. Excessive agitation causes turbulence, affecting cell structure, decreasing growth, and metabolite production. Conversely, insufficient agitation causes sedimentation and cell death (Robles-Heredia 2014, Montoya 2021). Hydrodynamic stress due to mechanical agitation and bubble rupture in FBRs can affect the growth and metabolism of microalgae (Arguelles et al., 2018; Alishah et al., 2019).

In this work, the variation of cell growth, nitrogen consumption, lipid productivity, and biodiesel quality was analyzed as a function of the type of FAME of *Chlorella vulgaris*, by the effect of hydrodynamics in column photobioreactors (FBRC), varying aeration flows at (0.75, 1.25, 1.75, 2.25) VVM and continuous white light; in addition to the cut-off rate to check for the probable presence of hydrodynamic stress. Hence, the interest in experimenting if at higher aeration flow and with discrete illumination, greater amounts of biomass and lipid production could be achieved in the microalgae.

Method

With the following methodological process, different determinations were carried out to know the variation of cell growth, nitrogen consumption, lipid productivity, and biodiesel quality as a function of the type of methyl ester components present FAME of *Chlorella vulgaris* in column photobioreactors (FBRC), alternating aeration flows of (0.75, 1.25, 1.75, 2.25) VVM and continuous white light.

Strain adaptation

The strain comes from the Ensenada Center for Scientific Research and Higher Education (CICESE), Mexico. It was acclimatized for 30 days in 250 mL Erlenmeyer flasks, for illumination cold white light was used with fluorescent lamps, with emission at 100 µmol m^{-2} s^{-1}, continuously. Transfers were made to 250 mL Erlenmeyer flasks.

Culture medium

For the medium at 90 mg L^{-1} of N-NH_4^+ (C_{90}), 3 mL of nutrients were added per L of water with the following composition: 7 mg NaCl, 4 mg $CaCl_2$, 2 mg $MgSO_4$ -7H_2 O, 15 mg KH_2 PO_4 , 115.6 mg NH_4 Cl, dissolved in 1L of water, similarly trace metals and vitamins were added considering the technique for f/2 culture medium (Guillar and Ryther 1962), sterilization was in autoclave at 120º C and 30 atm; once the culture medium was cold, 4 mL of vitamins were added per liter of medium (Robles-Heredia, 2014).

Inoculum

For the inoculum, 500 mL of solution were used at C_{90}. Maintaining constant agitation for 5 days to obtain a concentration of 1x10^6 cells mL^{-1} (cell x mL).

Cell growth

To verify cell development, every 24 h a 0.1 mm Neubauer Hematocytometer chamber was used to count the cells; with high cell densities, the sample was diluted 1:10 mL to facilitate counting, then the total number of cells counted was multiplied by a factor of 10 according to the dilution, taking into account equations (1 and 2) as appropriate (Ruiz-Marín et al., 2010):

$$Cc = \text{Cells counted} \times 10{,}000 \quad (1)$$

$$Ccd = \text{Cells counted} \times 10 \times 10{,}000 \quad (2)$$

Where: Cc represents the number of cells counted and Ccd represents the number of cells in dilution.

Biomass dry weight

Ten mL of the medium with microalgae were filtered using a constant weight filter, the filter with the biomass was introduced in an oven for 24 h at 130º C. Afterwards, the filter was placed in a desiccator to cool, the weight of the dry sample was obtained by weight difference, considering the sample volume used. The process was performed every 24 h until the end of the process (Robles-Heredia, 2014).

Nitrogen consumption

A 50 mL sample of culture medium was taken every 24 h, filtered, and 5 drops of H_2 SO_4 were added to fix nitrogen, then the sample was divided into 2 Erlenmeyer flasks with 25 mL each, 5 mL of borate buffer, and 4 drops of NaOH 6N were added. Boric acid indicator solution was added in 2 flasks, each of 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_2 SO_4 0.02 N until the solution turned from green to purple. The concentration of N-NH_4^+ was determined with the following equation (3) (Robles-Heredia, 2014):

$$N - NH_4^+ = \frac{Volume\ of\ acid\ spent\ x\ 0.02Nx14}{Sample\ volume} x1000 \quad (3)$$

Where: N-NH_4^+ represents ammoniacal nitrogen concentration; 0.02N represents normal sulfuric acid; 14 and 1,000 represent constant values.

Harvest

After the cultivation process, 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 preservation, then freeze-dried for 3 to 5 days (Ruiz-Marín et al., 2010).

Oil production

The modified method of Bligh and Dyer (1959) was used: 10 mg of lyophilized biomass were placed in tubes with thread, 4 mL of methanol, 2 mL of chloroform, and 0.5 mL of distilled water. This mixture was subjected to ultrasound for 15 minutes to break the cell wall and covered with aluminum foil, incubated for 24 h at 4 ºC. Afterwards, the aluminum foil was removed to submit them to ultrasound for 5 min, and they were centrifuged at 4,000 rpm for 10 min, the remaining liquid was transferred to new tubes with screw cap, and 4 mL of water were added for washing, The water of the tubes was removed with a Pasteur pipette, the chloroform was vaporized in a water bath, and 2 mL of 95% hydrochloric acid-methanol mixture was added. They were placed in a Hach DRB 200 digester for 1 h at 100ºC. Afterwards, they were wrapped again in aluminum foil to be kept refrigerated for 24 h at 4 ºC. Then, 3 mL of hexane were added and shaken to form a bi-phase from which the lower part was extracted with a Pasteur pipette. 4 mL of water were added to the tubes and shaken again. The water was extracted using a Pasteur pipette. They were wrapped again in aluminum foil and kept refrigerated for 24 h at 4 ºC. After the estimated time, they were vaporized in a water bath. 3 mL of hexane were added during the vaporization process before completing vaporization. The samples were transferred to vials and left to rest for 24 h. The lipid content was translated into lipid content in the tubes. Lipid content is translated to lipid composition (% ww^{-1}) on a dry w basis. And this, in turn, is translated to lipid productivity P_L (in mg L^{-1} d^{-1}) with the following equation (4):

$$P_L = \frac{w_2 X_2 - w_1 X_1}{t_2 - t_1} \quad (4)$$

Where: X_1 and X_2 is the mass concentration of dry biomass in the medium at time t_1 and t_2 (initial and final). w lipid content on a dry basis. P_L lipid productivity in units of mg L^{-1} d^{-1}

Obtaining fatty acid methyl esters.

The profile of fatty acid methyl esters (FAME) was obtained using an Agilent Technology 7890 gas chromatograph. 1 µL of the hexane-lipid solution was injected into the chromatograph with a flame ionization detector (FID) and a DB-23 separation column (60 m length, 0.32 mm ID, 0.25 µm thickness). Chromatographic conditions: T of the detector: 250 ºC; T of the injector: 250 ºC; Oven temperature program: 120 ºC for 5 min, increase the temperature at a rate of 10 ºC min up to 180 ºC, hold for 30 min, increase the temperature again at a rate of 10 ºC min up to 210 ºC and hold for 21 min (total 65 min); Carrier gas flow: 15 psi; Split: 1:100; Carrier gas: He high purity. A fatty acid methyl ester mixture standard (weight %). SupelcoTM 37 FAME component was used to identify the FAME components. The results of the experimental design were estimated with full factorial analysis of variance (ANOVA) (α: 0.05), using STATISTICA V7 software.

Assembly of the Column Photobioreactors (FBRC)

For the FBRC system, PET bottles with 2 L operating volume and others with 1 L volume were used; to avoid evaporation of the medium, distilled water was used to hydrate the injected air. The outgoing air was bubbled in water-chlorine to avoid external contamination; ¼ inch hoses were used for air injection. Cole Parmer vertical, transparent acrylic flowmeters for air control, Model ACRY-010052, air level 2-8 $Lmin^{-1}$, and strip-type dial; compressor of 2.5 Hp of power and 8.5 kg cm^{-2} of pressure to inject air. External illumination with cold white fluorescent light at 100 µmol m^{-2} s^{-1}. Figure 1 shows certain variables for hydrodynamic calculations.

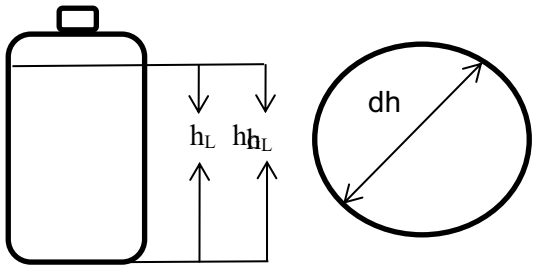

Figure 1. Bubbling column equipment (FBRC)

Where the indicated variables are as follows: 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 bubbling column (m); Ac is the cross-sectional area of the column (m^2); and ρL is the density of the liquid.

Aeration

Four aeration rates (0.75, 1.25, 1.75, 1.75. 2.25) vvm per experiment were considered, with one response variable (lipid productivity), one configuration (FBRC), and two replicates.

To calculate the shear rate the column height data (h_G) were (0.21, 0.22, 0.23, and 0.24) for each aeration flow, respectively; the following data were considered as constants for all experiments: h_L = 0.205 m; do = 0.12 m; Ac = 0.036 m^2; ρL = 998kg / m^3.

Equation (5) is considered to calculate the total air flow corrected by the absolute pressure at the bottom of the reactor (Robles-Heredia, 2014).

$$F_g = F_a \left(\frac{1}{60}\right)\left(\frac{1}{1000}\right) \quad (5)$$

Where: Fg is the total air flow expressed in (m^3 s^{-1}) and Fa the supplied air flow in (L min^{-1})

Equations (6) and (7) were used to calculate the column sectional area (Ac) in (m^2) and the gas surface velocity (Ug), which is the gas flow per unit area within the system in (ms^{-1}), according to Babcock et al, (2002).

$$Ac = b\ x\ h \quad (6)$$

$$U_g = \frac{Fg}{Ac} \quad (7)$$

With Ug for bubble column, the shear rate (γ) expressed in (s^{-1}) is calculated with equation (8) valid in the range of $0.008 < U_g < 0.09$ ms^{-1}. (Cerri et al., 2008).

$$\gamma = 1000{U_g}^{0.5} \quad (8)$$

The retained gas ε is calculated with equation (9); it determines the percentage of gas or air retained inside the equipment by the increase in air volume when air is injected (Doran, 1995).

$$\varepsilon = \frac{h_G - h_L}{h_G} \quad (9)$$

Equation (10) is used to calculate the surface velocity of the liquid, which is the liquid flow per unit area within the system. It can be calculated for bubbling columns with a diameter between 0.1 and 7.5 m and $0 < U_g < 0.4$ ms^{-1} (Doran, 1995).

$$U_L = 0.9\left(gdoU_g\right)^{0.33} \quad (10)$$

Where U_L is the surface velocity of the liquid in (m s^{-1}), g is the acceleration of gravity in (ms^{-2}), do is the diameter of the column in (m), and U_g is the surface velocity of the injected gas or air.

Equation (11) calculates pneumatic power, power generated by the air injected to the equipment for agitation of the fluid inside the FBRC. (Doran, 1995):

$$\frac{PG}{VL} = \rho_L g U_g \quad (11)$$

Where: PG/VL is the pneumatic power in (Wm^{-3}), calculated with ρL, which is the density of the liquid in (kg / m^3), g is the gravity in (ms^{-2}), and U_g the surface velocity of the gas or air injected. The use of different aeration flows was with the purpose of establishing the best aeration conditions, defining Cutoff Rate (ϒ) in order to verify the effect on methyl ester fatty acids, cell development, biomass production, nitrogen depletion, and lipid productivity.

Results and discussion

The results obtained from this work are shown below.

Table (1) shows the hydrodynamic calculations of the FBRC at the proposed aeration rates.

Table 1
Hydrodynamic data

Team	Aeration rate (vvm)	F_a (Lm^{-1})	F_g x 10^{-5} (m^3 s^{-1})	U_g x 10^{-3} (ms^{-1})	ε (%)	U_L x 10^{-2} (ms^{-1})	PG/VL (Wm^{-3})	ϒ (s^{-1})
FBRC	0.75	1.5	2.50	0.694	2.38	9.57	6.80	26.34
	1.25	2.5	4.16	1.150	6.81	10.18	11.26	33.91
	1.75	3.5	5.83	1.620	10.86	11.40	15.86	40.25
	2.25	4.5	7.50	2.080	14.58	12.40	20.36	45.60

It can be indicated that the algal cells did not present any type of deformation to the aeration conditions raised; similar aspects were reported by (Shi et al., 2016; Sadeghizadeh et al., 2017); as observed in Table (1), the values obtained from the hydrodynamics of the FBRCs, as the aeration flows increased, all the hydrodynamic parameters also increased which indicated the close relationship in each of them, besides the possible affectation with the other cellular parameters raised in this work.

Table (2) indicates cell growth, specific growth rate (μ), and nitrogen consumption obtained at the different suggested aeration flow rates.

Table 2
Cell growth, specific growth rate (μ), and nitrogen consumption obtained at different suggested aeration flow rates

Team	Aeration rate (vvm)	Max cell growth * (cell×10^6 mL^{-1})	μ * (h^{-1})	Consumption* N-NH_4^+ (%)
FBRC	0.75	6.80 ± 0.03[a]	0.0230± 0.09[a]	65.00± 0.08[a]
	1.25	3.10 ± 0.13[b]	0.0170± 0.06[b]	53.00± 0.07[b]
	1.75	2.51 ± 0.12[b]	0.0165± 1.70[b]	50.00± 0.93[b]
	2.25	2.49 ± 0.03[b]	0.0160± 0.50[b]	47.00± 0.05[b]

Note: * Different letters in the same column indicate significant differences according to Tukey test ($p \geq 0.05$); (± Standard deviation).

On the other hand, according to the maximum cell growth values in Table (2), it can be indicated that in relation to cell growth at 0.75 vvm, cells adapted to increase their development, reaching 6.80 x 10^6 cell mL^{-1} and the highest specific growth rate μ (0.023 h^{-1}); however, as the aeration rate was increased, cell growth was reduced more than 50% (3.10 x 10^6) cell mL^{-1} as well as the specific growth rate in all experiments. It can be mentioned that the obtained results of growth and nitrogen removal (N-NH_4^+) were well below other works (Kee-Lam et al., 2016; Sadeghizadeh et al. 2017, Anto et al., 2020; Montoya, 2021).

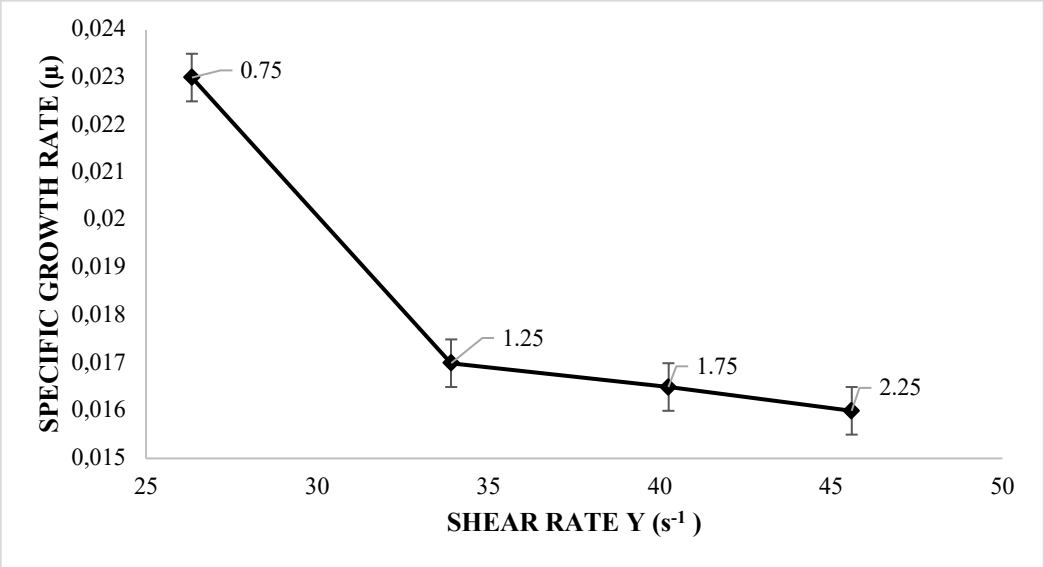

Figure 2. Growth rate μ *vs.* shear rate $\dot{\gamma}$

According to Figure 2, it can be seen that as the aeration flow increased, the values of the shear rates increased in each experiment, which may indicate that there was a direct relationship between these two parameters; however, the opposite occurred with the specific growth rate (μ). Therefore, it can be inferred that there was an inversely proportional relationship with these two aspects, the higher the aeration, the higher the shear rate ($\dot{\gamma}$), but lower the cell growth rate (μ); thus, it can be considered that the optimal air flow would be at 1.25 vvm to perform other experiments, depending on the product to be obtained, considering other factors of operation or illumination.

Table (3) shows the data of biomass dry weight, lipid content, and lipid productivity in *Chlorella vulgaris* at the proposed aeration rates.

Table 3
Dry biomass X, lipid content w, and lipid productivity P_L at the proposed aeration rates.

Team	Aeration rate (vvm)	X ($g\ L^{-1}$)	w_{max} (%ww^{-1})	P_L maximum ($mg\ L^{-1}\ d^{-1}$)
FBRC	0.75	0.295±0.056[a]	11.54 ± 0.03[a]	3.99 ± 0.026[a]
	1.25	1.395±0.010[b]	14.28 ± 0.02[b]	15.92 ± 0.019[b]
	1.75	0.430±0.041[a]	11.11 ± 0.02[a]	7.28 ± 0.016[a]
	2.25	0.405±0.050[a]	10.00 ± 0.04[a]	1.58 ± 0.016[a]

Note: [*]Different letters in the same column indicate significant differences according to Tukey test ($p \geq 0.05$); (± Standard Deviation)

Generally speaking, in this work, the values achieved for productivities were low, compared to other reports (Al-Ameri and Al-Zuhair 2019; De Jesus et al., 2019). However, at 1.25 vvm, the highest lipid productivity was observed (Table 3); even though at 0.75 vvm the highest cell growth was obtained, it can be indicated then that the cells at 1.25 vvm resented the degree of agitation, probably a slight stress occurred due to shear rate, which induced them to produce higher lipid content, increasing productivity. However, as the aeration rate increased, unfavorable conditions for lipid production were presented. Other reports indicate data of 1.2 g L^{-1} of biomass at different degrees of agitation, using light intensities greater than or close to 300 µmol m^{-2} s^{-1} (Chiu et al., 2008, Montoya, 2021), while Pham et al., (2017) report 1.35 g L^{-1} of biomass at 0.3 vvm, with illumination of 300 µmol m^{-2} s^{-1}; so, it can be inferred that the amount of light provided favors more in obtaining biomass than aeration. Similarly, other studies have reported that at illumination higher than 150 µmol m^{-2} s^{-1}, higher biomass increases were achieved (Jiang et al., 2016; Kim et al., 2015; Alishah et al., 2019; Castellanos et al., 2020).

Table (4) shows the main fatty acids of methyl esters produced by the microalgae, taking into account the culture conditions proposed.

Table 4
Main fatty acids of methyl esters produced by microalgae (in % of 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.a.	0.21(±0.11)	0.44 (±0.005)
C21:0	n.a.	n.a.	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.a.	n.a.	n.a.
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.a.	n.a.	n.a.	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.a.	n.a.	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]

Note: *Percentages in weight, different letters in the same row indicate significant differences according to Tukey test ($p \geq 0.05$); n.d.=not detected, (± Standard deviation).

The fatty acid methyl ester compounds (Table 4) that predominated were polyunsaturated (PUFA) since they were present at 0.75 vvm, 1.75 vvm, and 2.25 vvm; however, at 1.25 vvm, a greater presence of saturated fatty acids (SFA) was reached. The elements in higher amount were C12:0; C20:5N3; C24:1; C 22:0; C22:2, similarly to those reported by: (Al-Ameri and Al-Zuhair 2019; Alishah et al., 2019).

The analysis of variance showed that there was no significant difference between SFA and PUFA; similarly, the monounsaturated (MUFA) at 1.25, 1.75, and 2.25 vvm did not reveal significant differences ($p \geq 0.05$), while at 0.75 the value of MUFA was slightly higher. From the fatty acids obtained, it can be said that the increase in aeration in FBRCs favored higher PUFA production, especially at 1.75 vvm and 2.25 vvm, contributing to the characteristics of the biofuel; it is recommended that the composition of the lipids be long-chain fatty acids, with low degree of unsaturation to avoid toxic emissions and improve fuel properties such as cetane number (CN) and oxidative stability (OS) (Knothe, 2010, Basto-Flores et al., 2022).

Considering that within the main properties of fuels is the CN (cetane number), which in a dimensionless way describes the ignition quality of a fuel and is related to the

ignition delay time experienced by a fuel (Arguelles et al., 2018); according to the FAME analysis, due to the higher amounts detected of PUFA in the experiments, it can be indicated that biodiesel would be of low quality considering the low presence of saturated fatty acids.

Conclusions

From the above, it can be concluded that the aeration rate at 0.75 vvm was the most appropriate to obtain greater cell growth, but it should be noted that at 1.25 vvm there was a greater increase in lipid content, and therefore in lipid productivity. The working regime was in the homogeneous bubbling flow range due to the characteristics of the reactor and the surface velocity of the gas.

According to the conditions proposed in relation to cell growth, it would be convenient to manage an aeration rate of 0.75 vvm; on the other hand, in order to obtain greater lipid production, the aeration rate would be 1.25 vvm; therefore, a culture process could be considered, first growing the cells and then to conditions with agitation of 1.25 vvm to continue the culture, taking into account the concentrations of nutrients, the type of lighting, and verifying the productivity.

Importance of the study, strengths and weaknesses

This study was carried out with the purpose of knowing and interpreting the effect of aeration and some hydrodynamic parameters such as shear rate (γ) on the production of fatty acids of methyl esters (FAME) mainly, together with the fact of determining in the same way if these changes in aeration benefit other parameters such as cell growth, nitrogen consumption, lipid productivity, besides using correlations to determine in function of the FAME the quality of biodiesel, according to the type of components of methyl esters present at the proposed aeration flows.

In relation to the strengths, it can be indicated that there is an adequate methodological approach to calculate the hydrodynamics in the photobioreactors and other determinations, the knowledge to be able to interpret the results obtained, in addition to having the equipment to carry out the experimental part; regarding the areas of opportunity, it can be pointed out that it is necessary to continue experimenting, considering other operational factors, consider greater analysis of the determinations, and go deeper into these.

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