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Biomass and lipid production from *Chlorella vulgaris* UTEX 26 cultivated in 2 m^3 raceway ponds under semicontinuous mode during the spring season

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GRAPHICAL ABSTRACT

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ABSTRACT

A *Chlorella vulgaris* UTEX 26 semicontinuous culture was implemented in 2000 L raceways with M medium during spring season at greenhouse conditions. Areal biomass productivities between 20 and 26 g m^{-2} d^{-1} were reached on the third day. The maximal areal lipid productivity obtained was 6.1 g m^{-2} d^{-1} and an increment in the saturated fatty acids (SFA) proportion (C14–C18) was favored in comparison with the fatty acids obtained with M medium in photobioreactors of 1 L and photoperiod light:darkness 12:12 h. After the eighth day of the culture or biomass concentrations above 0.25 g L^{-1}, the microalgal cultures were prone to contamination by ciliates and amoebae, due to the sugars excreted by *C. vulgaris* UTEX 26. The periodical addition of NH_4HCO_3 to the microalgal culture maintained the ammonium concentration between 25 and 50 mg L^{-1}, which contributed to diminish the contamination risks by protozoa.

1. Introduction

The raceway (RW) reactors have offered a low cost microalgal culture systems to produce nutritional supplements, aquaculture feeding and biofuels among others, as well as wastewater treatment, nitrogen fixation and CO_2 mitigation (Hadj-Romdhane et al., 2012; Mendoza et al., 2013a). Microalgae have been taken into consideration as a feedstock for renewable biofuels production, such as bioelectricity, methane, biohydrogen, bioethanol and biodiesel (Ramanna et al., 2014; Chew et al., 2017; Fernández-Linares et al., 2017). However, microalgal biomass production to obtain biofuels is not economically feasible yet, whereby it is necessary to use high-productivity and economical

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reactors, as well as the use of economical, sustainable and available nutrients.

The RW reactors are the engineered approach most frequently employed for the commercial microalgal cultures (Morales-Amaral et al., 2015; Murphy et al., 2015; Yang et al., 2016), there are some culture conditions, like temperature and light irradiance variations, that are very difficult to control under outdoor conditions (Borowitzka and Moheimani, 2013). Moreover, contamination by protozoa, ciliates and other bacteria is a risk to microalgal production in the open systems. These organisms compete with the microalgal culture for sunlight and substrates (Lam et al., 2018). Grazers like ciliates, rotifers, copepods and cladocera are larger in size than microalgae (Wang et al., 2013) and they consume algae rapidly (Day et al., 2018, 2012), which leads to a significant decrease of the biomass yield. Nevertheless, to improve the microalgae production in RW, the selection of a suitable culture medium for each species of microalgae, as well as the culture mode, i.e., continuous, semicontinuous or sequenced batch must be considered. Additionally, modifications like baffles and flow deflectors in RW can be implemented to improve the microalgal biomass production (Huang et al., 2015). Several studies have been focused on implementing a swing gas aerator to generate small bubbles for improving mass transfer coefficient and microalgal growth rate in raceway ponds. Improvement of paddlewheel efficiency has also been considered in order to minimize energy costs (Yang et al., 2016).

The conventional culture media are not specific for a particular microalga. The Bold's Basal Medium (BBM) contains 0.25 g L^{-1} of $NaNO_3$ as the sole nitrogen source and is widely used for culturing several kinds of microalgae (Blair et al., 2014). Meanwhile, Highly Assimilable Minimal Growth Medium (HAMGM) was designed for *Chlorella vulgaris* culturing in an air-lift photobioreactor (PBR) under continuous mode (Hadj-Romdhane et al., 2012). HAMGM medium contains 1.099 g L^{-1} of NH_4HCO_3 as the nitrogen and carbon sources. Particularly, the M medium was designed in our research group through three stages of optimization, including the response surface method in order to enhance the biomass and lipid production simultaneously from *C. vulgaris* UTEX 26 cultivated in PBRs of 1 L and photoperiod of 12 h of light: 12 h of darkness. Thus, the M medium improved by 40% the biomass production (0.73 g L^{-1}) compared to the BBM. The lipid concentration in the M medium was 85% higher than the one obtained in HAMGM medium (Ramírez-López et al., 2016). An optimal medium formulation is required to ensure sufficient provision of nutrients to accomplish the maximal growth rate, and to produce biofuels at higher efficiencies (Schenk et al., 2008).

On the other hand, the RWs show low biomass productivities compared to the air-lift PBRs (Fon Sing et al., 2013). The main factors that influence productivity in RW are: a) The poor mixing and plug flow that occurs in the direction of flow, which leads to a low CO_2 solubility and O_2 accumulation. b) The average annual irradiance level and 3) The prevailing temperature. The culture temperature strongly affects the algal biomass productivity; CO_2 deficiency and alkalinization of the medium (pH control problems). The light intensity declines with depth and biomass concentration and restrain the specific microalgal growth rate. Another problem with RW is the contamination by rain, dust and zooplankton (grazers, bacteria and fungi) (Chisti, 2007, 2016; Lam et al., 2018). Considering all of the above, is important to highlight that the nutrients employed for the microalgal culture in a RW system should be sufficient to maintain the microalgal growth.

The main goal of this work was to evaluate the productivities of biomass and lipids of *C. vulgaris* UTEX 26, which was cultivated with M medium and semicontinuous mode in 2000 L RW during spring season at greenhouse conditions.

2. Materials and methods

2.1. Microalgal strain and inoculum growth conditions

The *C. vulgaris* UTEX 26 strain was acquired from the *Culture Collection of Algae*, Texas University (Austin, Texas, EE.UU.). *C. vulgaris* UTEX 26 was grown autotrophically in M medium (Ramírez-López et al., 2016) in RW of 200 and 2000 L. The composition of the M medium was (g L^{-1}): NH_4HCO_3, 0.45; $MgSO_4{\cdot}7H_2O$, 0.135; KH_2PO_4, 0.130; K_2HPO_4, 0.040; $(NH_4)_2HPO_4$, 0.100; $CaCl_2{\cdot}2H_2O$, 0.025; Na_2EDTA, 0.0375; $FeSO_4{\cdot}7H_2O$, 0.00374; $ZnSO_4{\cdot}7H_2O$, 0.0099; H_3BO_3, 0.00856; $MnCl_2{\cdot}4H_2O$, 0.00198, $CuSO_4{\cdot}5H_2O$, 0.00118, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.00071; $CoCl_2{\cdot}6H_2O$, 0.00060.

2.2. Raceway pond cultures at greenhouse conditions

The inocula for 2000 L RWs were obtained from microalgal cultures grown in 200 L RWs, consisting of 2.05 m channels, each one of 0.25 m wide and connected by 180° bends at both ends to provide a total surface area of 1.2 m^2. Experiments were performed in two 2000 L RWs; the dimensions of each one of the channels were 3.1 m long and 0.75 m wide, connected by 180° bends at both ends to provide a total surface area of 6.37 m^2. The culture medium depth was 0.18 m and mechanical agitation was provided by two paddle wheel systems (67 × 30 cm each paddle) at a superficial flow velocity of 30 cm s^{-1}. All open systems were conducted at greenhouse conditions in Mexico City (19 N 30′ 50.24″, 99 W 7′ 34.45″).

Microalgal cultures were performed at semicontinuous mode, completing 5 cycles (10–14 d $cycle^{-1}$), for 62 days during spring season (March 23–May 24). The working volume of the 2000 L RWs was 1100 L. During the harvesting, half of the culture volume was removed and replaced with the same volume of fresh culture medium. The water losses due to evaporation were quantified and replaced periodically. Light irradiance and greenhouse temperature were recorded with a data logger T&D RTR-500.

Samples were taken on days 0, 3, 6, 10 and 14 during the microalgal culture according to each culture cycle length. The samples taken were analyzed for biomass, ammonium and nitrate concentrations. Lipids in biomass were determined on days 6, 10 and 14 of each culture. Ammonium concentration was maintained between 25 and 50 mg L^{-1} to minimize and avoid contamination by protozoa (ciliates and amoebae).

2.3. Analytical methods

2.3.1. Biomass and lipid determination

The dry biomass, quantitative lipid and fatty acids methyl esters (FAMES) determinations were carried out according to Ramírez-López et al. (2016). The samples were analyzed in triplicate.

2.3.2. Microalgal culture parameters

Ammonium and nitrate concentrations were determined colorimetrically (Ramírez-López et al., 2016). All the analyses were carried out in the culture supernatant obtained by centrifugation at 3823 × *g* for 10 min (Hermle Z306, Germany). The culture temperature, pH and dissolved oxygen (DO) in the microalgal cultures were continuously determined with Hydrolab DS 5 probe (Hach).

2.4. Data analysis

All experiments were carried out in triplicate. The results were submitted to an ANOVA analysis ($p < 0.05$) to identify the relative significance of each treatment using Minitab 17 statistical software.

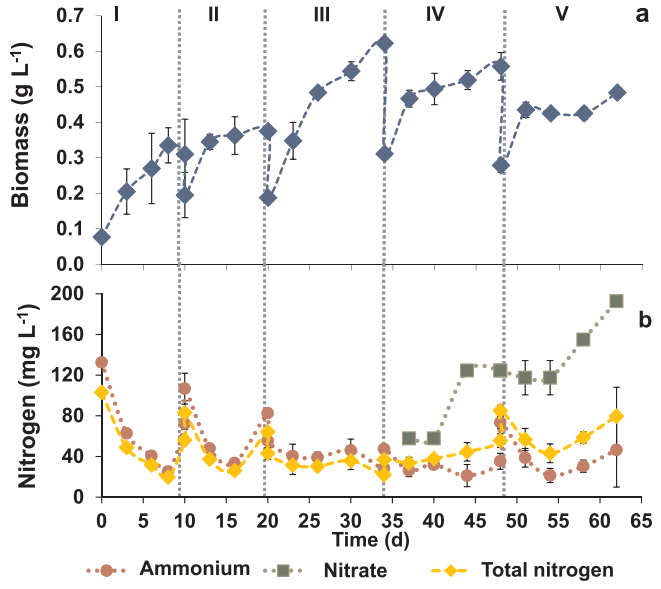

Fig. 1. a) Biomass production of *C. vulgaris* UTEX 26 with M medium in 2000 L RW at greenhouse conditions, during the five culture cycles (I, II, III, IV, V). b) Profiles of ammonium, nitrate and total nitrogen concentrations during the five culture cycles (I, II, III, IV, V).

3. Results and discussion

3.1. Semicontinuous culture of C. vulgaris UTEX 26 in 2000 L raceway pond system

In this section the evolution of the semicontinuous culture of *C. vulgaris* UTEX 26 in 2000 L RW systems (during five culture cycles) using M medium is presented. The culture cycle I started with a biomass concentration of 0.08 g L^{-1}, reaching the highest biomass concentration of 0.34 g L^{-1}, on day 8 of the microalgal culture (Fig. 1a). During this culture cycle, cellular agglomerates of *C. vulgaris* UTEX 26 were observed. These cellular agglomerates could be due to the excretion of organic metabolites, mainly carbohydrates, which was previously demonstrated in 1 L PBRs in which the carbohydrate concentrations were 25–40 mg L^{-1}. It has been reported that carbohydrates are the products mostly excreted by phytoplankton (Lee et al., 2009; Hadj-Romdhane et al., 2013) although there is evidence on the microalgae production of exopolysaccharides such as uronic or pyruvic acids, which are responsible for cell adhesion (Lee et al., 2009). Additionally, *C. vulgaris* JSC-7 produces a polysaccharide with phosphodiester (PO_2^-), hydroxyl and carboxyl groups, which serves as binding sites for autoflocculation (Alam et al., 2014). This kind of polysaccharides could be associated with the cell wall of *C. vulgaris* UTEX 26 and cause the microalgal agglomerates. In the same way, it is possible that the RWs hydrodynamics had favored the microalgal agglomeration.

Microalgae such as *C. vulgaris* present a preference for NH_4^+ as the nitrogen source (Hadj-Romdhane et al., 2012; Ramírez-López et al., 2016). This is due to the passive diffusion of NH_4^+ into the cell whereas the NO_3^- uptake requires membrane transporters and ATP (Liu et al., 2015). In this work, the concentration of NH_4^+ decreased 50% on the third day of the first culture cycle (Fig. 1b); however, only 0.13 g L^{-1} of biomass was produced, which does not explain the decrease of the initial concentration of NH_4^+ by assimilation. Most likely, a part of NH_4^+ was removed from the system by stripping, particularly when the pH of the microalgal culture oscillated between 8 and 8.7. As the pH increases, the dissociation equilibrium of dissolved ammonia favors the formation of the gaseous NH_3, which is easier stripped than the NH_4^+ form, the NH_4^+ predominates at pH values bellow 9.25 (pK_a = 9.25). Markou et al., (2014) reported higher ammonia losses (higher as 80%) when total ammoniacal nitrogen concentrations increased in the medium of *Arthrospira platensis* and ammonia losses were the highest when the initial pH was 10. Morales-Amaral et al. (2015) reported losses of 40% of initial NH_4^+ by stripping in a raceway system with a medium at a pH of 8.0, which was controlled by the addition of CO_2. Considering the above, in order to minimize the loss and ensure the NH_4^+ presence in the culture medium during most of the culture cycles, NH_4HCO_3 was gradually supplemented through the cycles, beginning at the second culture cycle, maintaining the NH_4^+ concentrations between 25 and 50 mg L^{-1} in the culture medium. Considering the chemical equilibrium between NH_4^+ and NH_3, at 20 °C, pH of 8.5 and without stripping, the NH_3 concentrations may have been between 4.4 and 8.8 mg L^{-1}. This strategy also contributed to the decrease of the risk of contamination by protozoa and rotifers, such as occurred in previous batch cultures of *Chlorella protothecoides* carried out by the working group, who used BBM medium maintaining the pH lower than 9.0, bubble column of 5 L and 50 mg L^{-1} of NH_4^+ for the control of contamination. Méndez and Uribe (2012) reported the application of doses of 60 mg L^{-1} of urea or 100 mg L^{-1} of NH_4HCO_3 (equivalent to 22.7 mg L^{-1} of NH_4^+) in the outdoor *Arthrospira* sp. culture for the control of *Branchionus* sp. and *Amoeba* sp. Additionally, Schlüter and Groeneweg (1985) cultivated *Scenedesmus costato-granulatus* and found that the rotifers (*Brachionus rubens*) died within two days with NH_3 concentrations over 6 mg L^{-1}, which is close to the NH_3 concentrations employed in this work.

In the second cycle, on the third day of microalgal culture, coenobium of *Scenedesmus* sp. were observed, and on the 9th day there was a contamination by ciliates in one of the RWs, for this reason it was decided to add 65 mg L^{-1} of NH_4^+ in the form of NH_4HCO_3 in order to eliminate the ciliates. On day 10 the biomass production (0.38 g L^{-1}) was 22.6% higher than the biomass obtained in culture cycle I, therefore, it did not increase significantly (Fig. 1a).

The accumulation of organic compounds excreted in the culture medium increases the risk of contamination (Moheimani y Borowitzska, 2006; Hadj-Romdhane et al., 2013). Protozoa appeared in the *C. vulgaris* UTEX 26 cultures when the microalgal biomass was of 0.35 g L^{-1} and this is related to the presence of carbohydrates in the culture medium. The presence of excreted carbohydrates was previously determined in *C. vulgaris* UTEX 26 cultures carried out in 1 L PBR under controlled conditions at which the biomass concentration was of 0.49 g L^{-1} and the excreted carbohydrates concentration was of 25–40 mg L^{-1}.

On day 14 of the culture cycle III (34 days of semicontinuous culture), the highest biomass production (0.62 g L^{-1}) was reached (Fig. 1a). This biomass production was similar to the biomass concentration reported by Wang et al. (2014), who cultured *Chlorella pyrenoidosa* with an optimized F-Si medium, in 0.35 m^2 bubbling open ponds (bubbling air supplemented with 2% CO_2 at 22.5 L min^{-1}) at 45 L as the working volume and 14 cm depth and outdoor conditions. In this culture cycle, both *C. vulgaris* UTEX 26 and *Scenedesmus* sp. were present. It is important to highlight that in this work open systems were used. Despite of being located inside a greenhouse, the RWs were subject to biological contamination, water evaporation and fluctuations in light irradiance and temperature, therefore, this work provides information on the performance of the *C. vulgaris* UTEX 26 cultures amended with M medium in open systems, which will basically serve to improve the microalgal culture strategies at large scale.

In the last four culture cycles, the volumetric and areal biomass productivities decreased considerably after the third day of the microalgal culture, when the biomass concentration was of 0.33 g L^{-1}. Biomass productivities could have been affected by the cell density, which avoids the passage of light through the culture, by the shadow effect of upper cells. In this regard, in the culture cycles II and III, on the third day, an areal biomass productivity of 20.1 g m^{-2} d^{-1} was obtained, it was 136% higher than the biomass productivity reported for a RW with the same configuration that was used in this work (Huang

et al., 2015). Also, it was 38.6% higher than the biomass productivity obtained in a modified RW with inclined baffles (at bottom) and flow deflectors, in which *C. pyrenoidosa* was cultivated for three days (Huang et al., 2015). Furthermore, the areal biomass productivity obtained in these culture cycles on third day was 2.4 times higher than the areal biomass productivity obtained with *Nannochloropsis oculata* in 200 L RW, which were operated under semicontinuous mode at greenhouse conditions (Millán-Oropeza and Fernández-Linares, 2017). The aforementioned could indicate that the semicontinuous culture cycles for *C. vulgaris* UTEX 26 should not be longer than three days to obtain high areal biomass productivities.

In the culture cycle IV, the biomass of *C. vulgaris* UTEX 26 decreased due to the appearance of amoebas; however, there was presence of *Scenedesmus* and *Chlorococcum*. The presence of these microalgae exerted an effect on the color of the microalgal culture, which was darker. In this culture cycle, the biomass production was significantly lower ($p < 0.05$) compared with the culture cycle III. On third day, an areal biomass productivity of 27 $g\,m^{-2}\,d^{-1}$ was obtained, it was 12.5% higher than the areal biomass productivity obtained by Morales-Amaral et al. (2015), who cultivated *Scenedesmus* sp. in a RW under different conditions, i.e., 32 m^2 and 4.4 m^3 of total culture volume with an aerated tank (1 m depth) at semicontinuous mode and a daily renewal rate of 20%. During this culture cycle the NO_3^- concentrations in the culture medium increased (Fig. 1b), which was due to nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter,* whose presence was proven by their growth in the Winogradsky media (Data not shown). During the culture cycles IV and V, a competition for the NH_4^+ between microalgae and nitrifying bacteria occurred, this was assumed by the increase in NO_3^- concentrations whereas the microalgal biomass did not exhibit the same behavior. It has been reported that in a culture medium with NH_4^+ concentrations higher than 0.018 $mg\,L^{-1}$, the NO_3^- assimilation is suppressed (Addy et al., 2017).

On the other hand, considering the coexistence of *Chlorococcum* and nitrifying bacteria during the last two culture cycles, a consortium that could be used for the NH_4^+ removal in wastewater could have been naturally integrated. In such scenario, the microalgae would provide oxygen for nitrification development, which has already been reported by Karya et al. (2013), who created a consortium with *Scenedesmus quadricauda* and nitrifying bacteria from activated sludge coming from a wastewater treatment plant.

Finally, in the last cycle and with a significantly lower biomass ($p < 0.05$) than that obtained in the culture cycles III and IV (Fig. 1a), *Chlorococcum* and *Scenedesmus* sp. predominated over *C. vulgaris* UTEX 26. In the culture cycles IV and V no ciliates or amoebas appeared unlike the culture cycles II and III, when the predominant microalgae was *C. vulgaris* UTEX 26, which could indicate that this microalgae is more susceptible to protozoa contamination.

Considering the biomass results obtained in the five culture cycles of this work and the days of appearance of protozoa, for *C. vulgaris* UTEX 26 cultures in 2000 L RWs with a working volume of 1100 L some suggestions could be implemented. For example, it may be necessary to diminish the time and the renewal rate of the microalgal culture to three days and to 15 or 20%, respectively, in order to ensure a greater light penetration and an increase in the biomass productivities. With this strategy, the accumulation of organic compounds excreted by microalgae, which causes the appearance of protozoa even at concentrations of NH_4^+ higher than 30 $mg\,L^{-1}$, would also decrease.

3.2. Lipid concentrations and fatty acid profiles

At the end of the first culture cycle (10 days), 277 $mg\,L^{-1}$ of lipids were obtained (Fig. 2). This was the maximal lipid concentration obtained in the five culture cycles, which corresponded to an areal lipid productivity of 6.1 $g\,m^{-2}\,d^{-1}$, this was 18.7% lower than the lipid productivity obtained with *C. pyrenoidosa* grown with an optimized F-Si medium, in 0.35 m^2 bubbling open ponds at outdoor conditions (Wang et al., 2014). Luo et al. (2016) reported lipid productivities of 13.42 ± 0.04 $mg\,L^{-1}d^{-1}$ with *Coelastrella* sp. cultivated in 40% of anaerobically treated swine wastewater. The productivity reported was 51.55% lower than the maximum productivity obtained in this work.

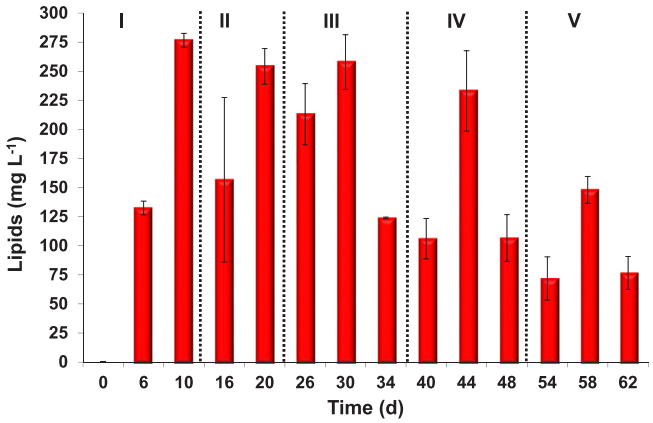

Fig. 2. Microalgal lipid production using M medium in 2000 L RW at greenhouse conditions during the five culture cycles (I, II, III, IV, V).

Table 1
Fatty acid profiles of the microalgal cultures grown with M medium on day 10 of culture, corresponding to the first four culture cycles in semicontinuous mode, in 2000 L RW at greenhouse conditions (% of total FAMES).

	Culture cycle			
	I	II	III	IV
Fatty acid	%			
C14:0	4.2	4.6	5.4	5.3
C16:0	52.7	44.9	41.7	40.1
C16:1	2.5	3.8	3.4	5.6
C18:0	29.1	26.6	23.2	17.9
C18:1	3.0	6.3	4.6	6.2
C18:2	1.8	4.0	3.3	3.9
C18:3n3	6.7	9.8	18.4	21.0
C14-C18	100	100	100	100
Unsaturated	14.0	23.9	29.7	36.7
Saturated	86.0	76.1	70.2	63.3

In the culture cycles II, III and IV, the highest lipid production was also presented on day 10 (Fig. 2) and there were no significant differences against the first cycle. In the culture cycle V, the lipid concentration was significantly lower ($p < 0.05$) compared to the first four culture cycles (Fig. 2) and *C. vulgaris* UTEX 26 no longer predominant in the microalgal culture.

Gas chromatograph analysis of lipids extracted from microalgal culture grown on RW with M medium showed a fatty acid profile that consisted of fatty acids with carbon chains ranging from C14 to C18 (Table 1) on day 10 of culturing, during the culture cycles I, II, III y IV. The main fatty acids were palmitic acid (C16:0) and stearic acid (C18:0), followed by oleic acid (C18:1) and linolenic acid (C18:3), which are suitable for obtaining biodiesel with good properties (Cho et al., 2015; Jusoh et al., 2015). *Chlorella sorokiniana* grown with wastewater at laboratory scale, showed a similar fatty acid profile, with carbon chain lengths between C14-C18 (Ramanna et al., 2014). Similarly, Millán-Oropeza and Fernández-Linares (2017) found that the most abundant fatty acids in *N. oculata*, cultivated in 200L RW at greenhouse conditions were C16:0 and C18:0 with an abundance of 37.2 and 27.1%, respectively.

The percentage of saturated fatty acids (SFA) increased up to 1.6 times (culture cycle I) than the SFA obtained with 1L FBRs (Ramírez-López et al., 2016). In culture cycle I, the SFA percentage was six times higher than the unsaturated fatty acids. The highest percentages of SFA

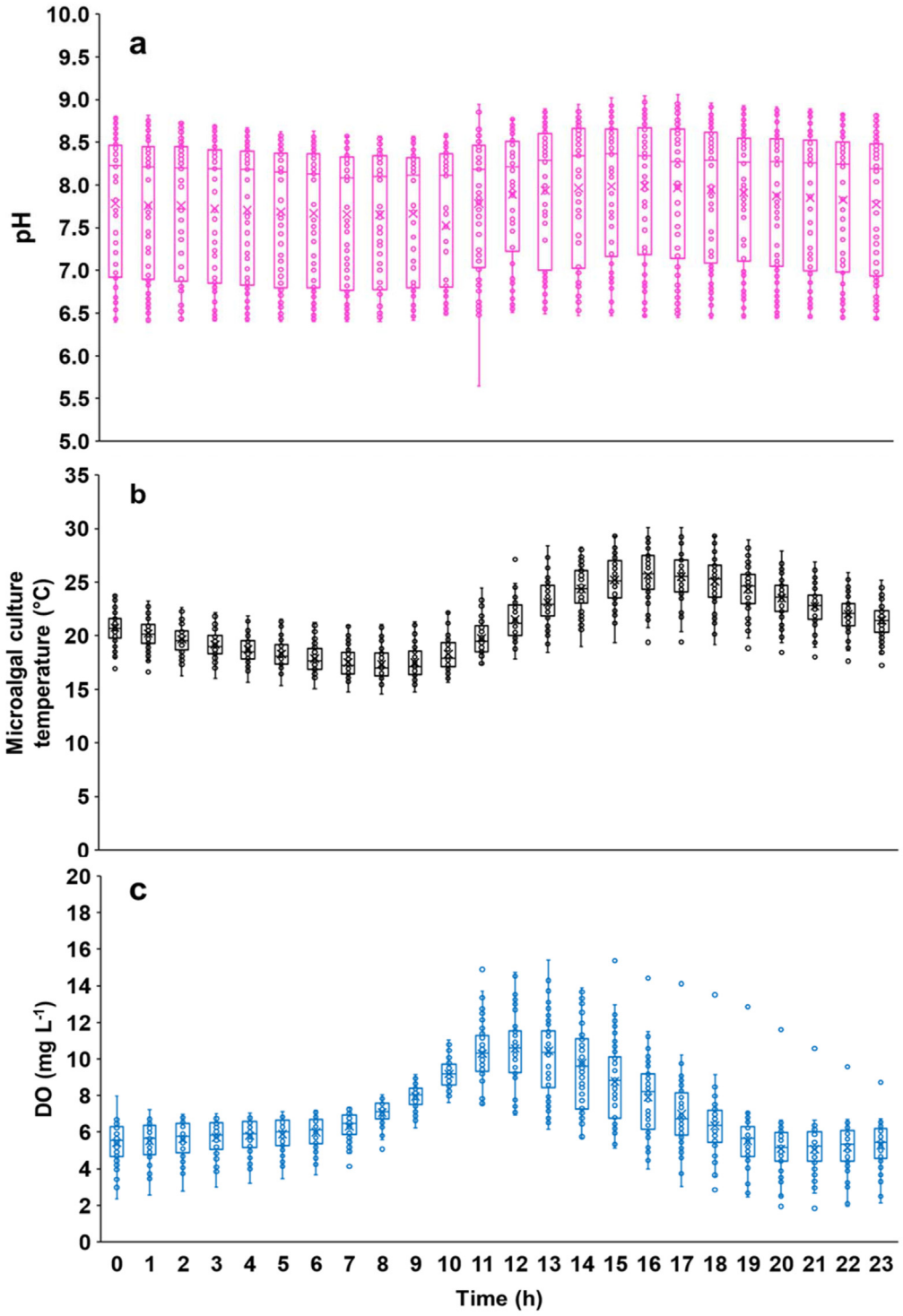

Fig. 3. a) pH values of the semicontinuous cultures of *C. vulgaris* UTEX 26 at greenhouse conditions during the five culture cycles (I, II, III, IV, V). b) Temperatures of the semicontinuous cultures of *C. vulgaris* UTEX 26 at greenhouse conditions during the five culture cycles (I, II, III, IV, V). c) Dissolved oxygen (DO) variation in the semicontinuous cultures of *C. vulgaris* UTEX 26 at greenhouse conditions during the five culture cycles (I, II, III, IV, V).

were obtained in the first two growing cycles (Table 1) and decreased as the seminontinuous culture progressed, which is related to the population transition and decrement of *C. vulgaris* UTEX 26.

Additionally, the presence of myristic acid (C14:0) in this work was determined in the culture cycles I-IV. Myristic acid was not identified when the microalgal strain was cultivated with M medium in 1 L FBRs at controlled conditions under artificial light, photoperiods and temperature (Ramírez-López et al., 2016). Nitrogen and phosphorus deprivation, salinity stress, carbon source, metal ions, pH and temperature are the most important and applicable environmental parameters on the algal lipid content and composition (Sajjadi et al., 2018). The controlled conditions of temperature, light and aeration of the *C. vulgaris* cultures in FBR of 1L were very different from those of the RW in greenhouse conditions, which had no temperature control and were subject to the circadian cycle, which in turn affects the pH as well as the availability of CO_2 and ammonium in the medium. The temperature not

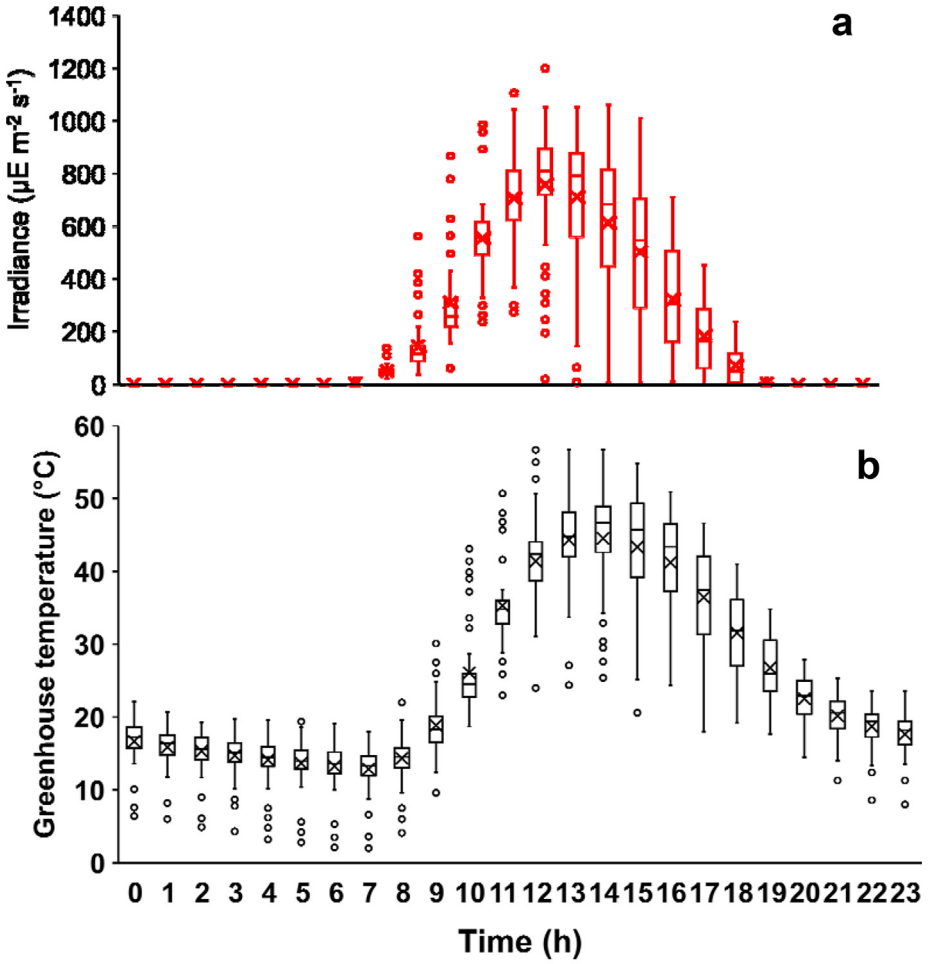

Fig. 4. a) Light irradiance inside the greenhouse during the five cycles of the semicontinuous cultures of *C. vulgaris* UTEX 26. b) Temperatures inside the greenhouse during the five cycles of the semicontinuous cultures of *C. vulgaris* UTEX 26.

only strongly influences the growth rates and carbon fixation, but also affects biochemical composition such as lipids, proteins and carbohydrates (He et al., 2018). Seasonal temperature fluctuations, daily temperature variations, and abrupt temperature variations can modify the growth conditions of microalgae and thus its productivities (Sajjadi et al., 2018). Roleda et al., (2013) reported the variation in the amount and lipid profile in *Thalassiosira pseudonana*, *Odontella aurita*, *Nannochloropsis oculata*, *Isochrysis galbana*, *Chromulina ochromonoides*, and *Dunaliella tertiolecta*, due to the temperature and the composition of the medium. Light intensity exerted only a minor impact on the fatty acid composition (Breuer et al., 2013; Liu et al., 2012; Pal et al., 2011). Fatty acid profiles in *C. vulgaris* under either continuous monochromatic illumination or wavelength shifts were not altered significantly with the change of the wavelength; however, total FAME was highly dependent upon light illumination (Kim et al., 2014).

Additionally, Deng et al. (2018) reported the absence of myristic acid when *Chlorella kessleri* UTEX 2229 was grown in mixotrophic cultures, under photoperiods of 4:20 and 0:24 light:dark with a light intensity of 90 µmol photons $m^{-2}s^{-1}$; however, under longer periods of illumination, myristic acid was detected in the lipid profile.

3.3. Variation of pH, temperature and dissolved oxygen (DO) in the microalgal culture and fluctuations of temperature and light irradiance in the greenhouse

Regarding the pH variation in the microalgal culture, in all the culture cycles, the highest pH values were recorded from 18:00 to 20:00 h, while the lowest values were in the morning, from 8:00 to 10:00 h (Fig. 3a). In the culture cycles I, II and III, pH values oscillated between 8.0 and 9.0, with daily average values of 8.5, which are within the interval for the optimal microalgal growth (Kumar et al., 2015). A different behavior was observed in the culture cycle IV, where the pH of microalgal culture decreased from daily average values of 8.0 (first days) to values of 6.5 at the end of the culture cycle. The pH decrease in the culture cycles IV and V was due to the nitrification process, in which the NH_4^+ was oxidized to NO_3^- and H^+ ions were released into the culture medium to form HNO_3, which is responsible for the acidification of the microalgal culture (Addy et al., 2017). The competition of microalgae and nitrifying bacteria for NH_4^+, along with the pH decrease in the second half of the culture cycles IV and V, could have affected the biomass production (Fig. 1a).

Regarding the microalgal culture temperature (Fig. 3b), the highest temperatures were recorded between 15:00 to 18:00 h, while the lowest temperatures were recorded between 7:00 to 9:00 h. On average, the temperatures ranged between 17.0 and 28.4 °C in all culture cycles, therefore, the global daily average temperature of the microalgal cultures was of 22.7 °C.

The average DO concentration for the first three culture cycles was of 8.5 mg L^{-1}, with a minimum between 5 and 7 mg L^{-1} at night. In the culture cycles IV and V, the average DO concentration decreased to 7 mg L^{-1} and the minimum oscillated between 2 and 5 mg L^{-1}, which was attributed to the O_2 consumption by nitrifying bacteria. On the other hand, the maximal daily DO concentrations (Fig. 3c) were similar to the DO recorded with *S. quadricauda* grown in 1 L PBRs with aeration

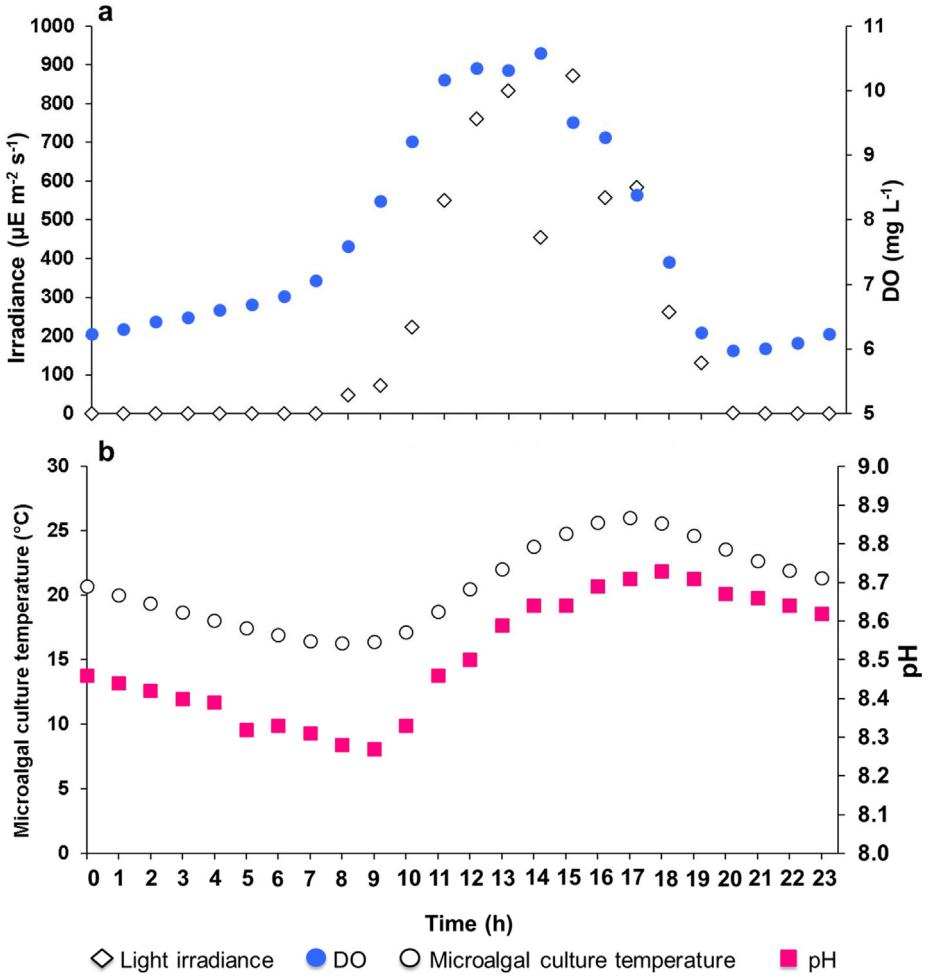

Fig. 5. Daily representation of the light irradiance variation, DO, culture temperature and pH during the *C. vulgaris* UTEX 26 culture, in 2000 L RW. Data correspond to April 15, 2016 (day 3 of the culture cycle III).

and mechanical agitation (Karya et al., 2013). Although these values of DO were up to 70% lower than the DO concentrations reported by Mendoza et al. (2013b) with a *Scenedesmus* sp. culture, in RWs with 100 m^2 and a sink of 0.59 m^3 for aeration enriched with 10 and 100% of CO_2. DO concentrations above 25 mg L^{-1} have been reported as critical for microalgal growth, since the biomass concentration decreases due to cellular damage by photo-oxidation and there is inhibition of photosynthesis by respiration (Jiménez et al., 2003; Kumar et al., 2015). It is unlikely that a cellular damage occurred in the microalgal cultures carried out in the present work because DO values were never higher than 16 mg L^{-1} during all the culture cycles.

The highest values of light irradiance inside the greenhouse were recorded between 12:00 and 16:00 h every day (Fig. 4a), and the daily maximal average of light irradiance inside the greenhouse was of 908.5 μE m^{-2} s^{-1}. The highest temperatures inside the greenhouse (Fig. 4b) corresponded to the period of the greatest light irradiance. The average global daily temperature in the greenhouse was of 31 °C.

In particular, to clearly appreciate the correlation between light irradiance, DO, temperature culture and pH, the third day of culture cycle III was chosen (April 15, 2016) (Fig. 5). In an entire day, it could be observed that the DO increased as the day progressed and the light irradiance increased. This demonstrated that there was a proportional relation between the light irradiance and the DO concentration in the microalgal culture (Fig. 5a). The DO concentration in the medium during the microalgal growth is related to the photosynthetic activity, a high photosynthetic activity favors high DO values, which is directly related to the light irradiance that the microalgal culture receives (Jeon et al., 2005). This same behavior was reported by Mendoza et al. (2013b) and Fernández et al. (2016) with microalgal cultures carried out in RW of 100 m^2 in Almeria, Spain. During the darkness period, in this work, the DO concentrations were close to 6 mg L^{-1}, whereas Mendoza et al. (2013b) reported values of 7.5 mg L^{-1} (O_2 saturation) during the darkness period in *Scenedesmus* sp. cultures in RW of 100 m^2 with a sink of 0.59 m^3 for aeration enriched with 10 and 100% of CO_2. Around 8:00 h, the DO concentrations close to saturation (7.5 mg L^{-1}) were recorded, which soon increased to reach a super-saturation with DO concentrations close to 10.5 mg L^{-1}, this was observed between 11:00 and 14:00 h (Fig. 5a). Then, the DO decreased as well as the light irradiance, coinciding with the period of maximal culture temperatures (25 °C) of all day, also causing the decrease of the O_2 and CO_2 solubilities.

The temperature and pH of the microalgal culture presented a behavior similar to the light irradiance and the DO concentration at different times of the day. The highest values were moved towards the afternoon, between 15:00 and 19:00 h, while the lowest values were recorded in the morning, between 6:00 and 10:00 h. Increments in pH and temperature of microalgal culture during the day were due to photosynthesis and solar radiation, respectively. Particularly, for the third day of the culture cycle III, the pH oscillated 0.4 units, while the culture temperature oscillated 10 °C (Fig. 5b). Fernández et al. (2016) cultivated *Scenedesmus almeriensis* CCAP 276/24 in a RW of 100 m^2 in Almeria, Spain, and obtained a proportional variation between the light irradiance, DO and pH of the microalgal culture. The increase and decrease of values of these parameters were presented at the same time

during the day, unlike what happened in this work, because the maximal pH values occurred when the light irradiance and DO began to decrease (Fig. 5). Slegers et al. (2013) reported maximum temperatures of the medium, in microalgal cultures carried out in Holland and in Algeria, when the light irradiance and the DO concentration began to decrease, the same effect was observed in the present work.

4. Conclusions

The utilization of M medium with NH_4^+ concentrations between 25 and 50 mg L^{-1}, as an operative strategy for a *C. vulgaris* UTEX 26 culture at pilot scale and semicontinuous mode, favored competitive areal biomass productivities (20–26 g m^{-2} d^{-1}, on the third day) and decreased the protozoa appearance. A shorter culturing time and renewal rate for the microalgal culture to three days and 15 or 20%, respectively, could ensure a greater light penetration and an increase in the biomass productivities. In regards of lipid production, an increment of the proportion of SFA (C14-C18) was favored.

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