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Growth of *Chlorella vulgaris* and *Nannochloris oculata* in effluents of Tilapia farming for the production of fatty acids with potential in biofuels

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The use of microalgae in wastewater treatment and its biotechnological exploitation for the production of biofuels is a potential environmental application. Some species of microalgae are notable due to their lipid composition and fatty acid profile suitable for biofuel production. During the present study, a factorial 2^3 experimental design was conducted, which assessed three factors: i) two species of microalgae (*Chlorella vulgaris* and *Nannochloris oculata*), ii) two types of culture media [wastewater of tilapia farming (WTF) and bold's basal medium (BB)], and iii) two types of lighting (multi-LED lamps and white light). Microalgae were inoculated in photobioreactors in 6 L of medium (WTF or BBM) at an initial concentration of 1.0×10^6 cells ml^{-1} at 20 ± 2°C. The highest average cell density as well as the highest productivity of biomass observed in the treatments was *C. vulgaris* treatment in BBM and multi-LED lighting (8.83×10^7 cells ml^{-1} and 0.0854 g l^{-1} d^{-1}, respectively). Although the majority of lipid productivity was obtained in the exponential phase of *N. oculata* cultivated in multi-LEDs in both treatments (BBM with 58% and WTF with 52%), cultivation of both species was generally maintained in WTF and were those that presented the major lipid productivity (2-18 mg l^{-1} d^{-1}) in comparison with those cultivated in BBM. Palmitic, stearic, oleic, linoleic, linolenic and eicosanoic (C16–C20) fatty acids were present in both species of microalgae in concentrations between 26 and 74%. Based on the results of the present study, we conclude that cultivation of *N. oculata* and/or *C. vulgaris* in WTF illuminated with multi-LEDs is an economic and sustainable alternative for biodiesel production because it can represent up to 58% of lipids with a fatty acid profile optimal up to 74% of the total fatty acids.

Key words: *Chlorella vulgaris, Nannochloris oculata*, production of fatty acids, wastewater of tilapia farming, production of biofuels.

INTRODUCTION

During the last few decades, an energy crisis associated with depletion of irreversible traditional fossil fuel sources is being recognized worldwide. We are aware that its use as a primary source of energy is unsustainable and

contributes to the accumulation of greenhouse gases, which causes global warming and a permanent source of atmospheric environmental pollution (Ahmad et al., 2011; Amaro et al., 2011). Likewise and with current trends of fossil energy consumption, worldwide oil reserves could be depleted by the year 2050 (Demirbas, 2011; Chen et al., 2013). Because of this, international trends of power generation and environmental protection are derived from the research and development of renewable, economically competitive and environmentally friendly alternative sources of energy (Ahmad et al., 2011; Chen et al., 2013; DeJong et al., 2013). Liquid fuels derived from plant matter (also called biofuels) are an alternative to the generation of sustainable energy. In comparison with other renewable forms (for example, wind, tidal and solar), these allow storage for long periods of time due to their chemical structure and can be used in addition to the traditional forms in engines and existing transport infrastructure after mixing to varying degrees with diesel oil (Singh and Gu, 2010; Amaro et al., 2011). The choice of biomass as feedstock for the production of energy depends on social, environmental, economic and industrial factors in addition to its availability and cost. However, generation of biodiesel from grain legume entails the use of large tracts of arable land and fresh water for cultivation. Also, there is the possible competition with food production of direct use to man or animal in addition to seasonal and geographical variations that also affect productivity as well as the use of herbicides and the consequent environmental pollution (Chen et al., 2013).

Therefore, production of biodiesel from microalgae is an important option that should be evaluated as an alternative for the generation of biofuels. This also seems to be a renewable source of fuel that can satisfy the global demand for transport fuels (Chisti, 2007; Demirbas, 2011) but also has the potential to generate large volumes of feedstock without affecting the food supply (Rosch et al., 2012). Microalgae as biofuel producers have different advantages such as high productivity, accumulation of lipids, and ability to grow in wastewater. In addition, microalgae have a higher productivity per area and have the ability to grow in non-arable lands with water unsuitable for agriculture, using CO_2 and other industrial waste (Delrue et al., 2012; Lohrey et al., 2012; Chen et al., 2013; Sánchez et al., 2015). A scarcely exploited alternative for the generation of microalgal biomass is waste water of production aquaculture, which may have as an advantage the biological water treatment and reinstatement into the aquaculture system. This allows the nutrition of microalgae using organic compounds (nitrogen and phosphorus) available in these effluents (Mata et al., 2010; Chávez-Crooker and Obreque-Contreras, 2010; Marinho-Soriano et al., 2011). It is reported that algae produce more lipids in a stress environment or under unfavorable conditions compared to optimal growth conditions. During optimal growth conditions, algae synthesize fatty acids mainly for esterification to glycerol in membrane lipids, which constitute ~5-20% of their dry weight. However, under conditions of stress, by limiting nitrogen or another component, microalgae have a very high production of lipids that can reach up to 77% of its dry weight (Mata et al., 2010; Kirrolia et al., 2013; Josephine et al., 2015).

The present study assessed the production of fatty acids in two freshwater microalgae *Chlorella vulgaris* and *Nannochloris oculata* cultured in Wastewater of Tilapia Farming (WTF) and Bold's Basal Medium (MBB) using two lighting systems in order to determine their potential for the generation of biofuels.

MATERIALS AND METHODS

Strains and growth medium

C. vulgaris (code: CLV2) and *N. oculata* (code: LB2194), were obtained from the collection of the Department of Aquaculture of the Center of Scientific Research and from the Center for Higher Education and Teaching (CICESE) and from the Collection of the University of Texas (UTEX), respectively. Strains were maintained in sterile BBM in flasks of 125 ml under controlled laboratory conditions (Bischoff and Bold, 1963; Nichols, 1973; Andersen, 2005) during a 24 h photoperiod of light without aeration and temperature of 18°C in a light chamber at 18.5 µmol m^{-2} s^{-1}. The large-scale culture of *C. vulgaris* and *N. oculata* was carried out in four 1000 ml Erlenmeyer flasks with 750 ml of sterile BBM and 100 ml of inoculum suspension (1.0×10^6 cells ml^{-1}) for reseeding. Lighting conditions were 24 h photoperiods with constant aeration at a temperature of 18°C in a light intensity of 79.88 µmol m^{-2} s^{-1}.

Photobioreactors, treatment systems

For the cultivation of microalgae, *C. vulgaris* and *N. oculata*, 16 photobioreactors were used, which consisted of 15 x 45 cm acrylic hexagonal units with a capacity of 8 L and closed completely by having the top drilled. Two 5 mm glass tubes were placed, the first to provide oxygen and homogenize cultivation using a pump. The second glass tube was used to take samples to monitor the cultivation. During the study, photobioreactors were placed in two structures in order to have two modules, which are illuminated individually through the following schemes: module 1 lighting the

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Abbreviations: BBM, Bold's basal medium; **WTF,** wastewater tilapia farming; **Nn,** *Nannochloris oculata*; **Ch,** *Chlorella vulgaris;* **LED,** multi-LED lighting; **WL,** white lights.

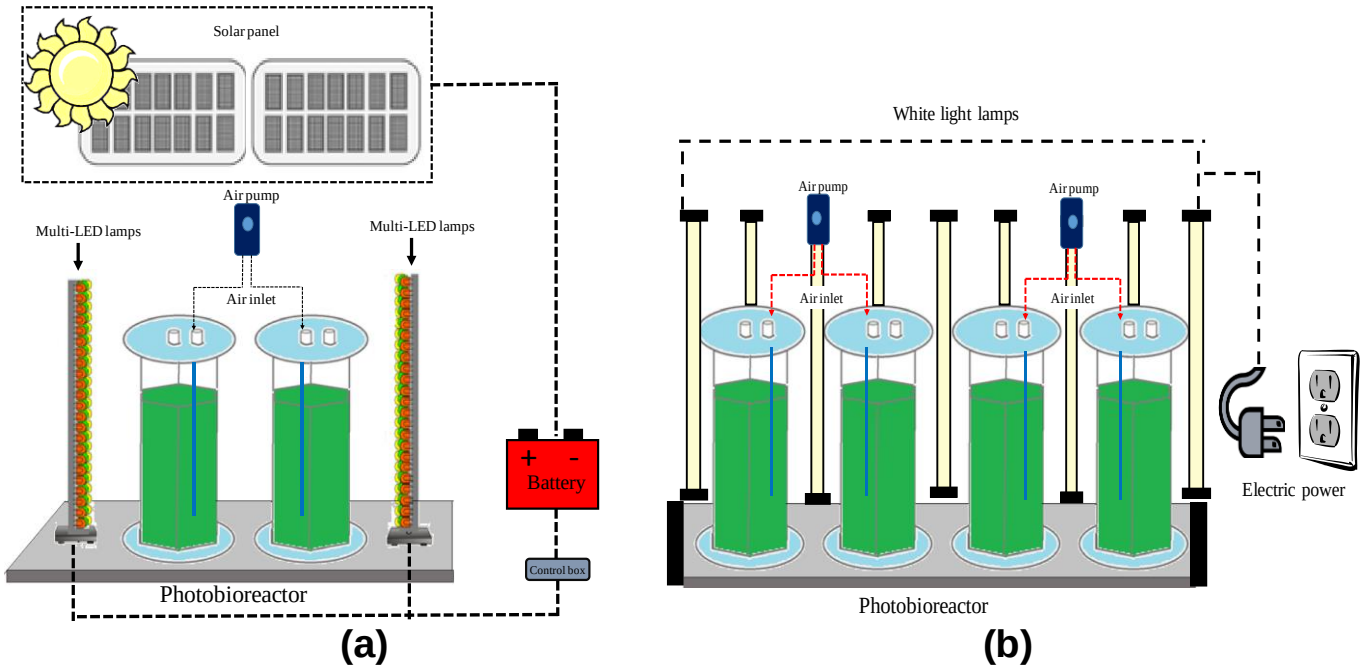

Figure 1. Types of lighting in farming systems. **(a)** Multi-LED lamps. **(b)** White light lamps.

photobioreactors generated from four multi-LED reflectors (RF-240HFS 30W) energized by two batteries (Surrette S-600 deep cycle 6V, 450 Ah) connected to five solar panels (polycrystalline 145 W modules placed in the upper part of the roof of the LLF). The second module consisted of four 40 W white lights energized with direct current (220 V). Both systems (Figure 1) maintained a light intensity of 79.88 µmol m^{-2} s^{-1}.

Experimental design, sampling and analyses

The experiment consisted of a 2^3 factorial design, where three factors were evaluated: A) type of microalgae, B) type of culture medium, and C) type of lighting at two levels each (8 treatments in total, with a replica by treatment). The experimental units (16 photobioreactors) were then added to 6 L of the microalgae culture medium corresponding to each treatment: eight photobioreactors with sterile BBM (control) prepared previously according to the specifications by Nichols (1973) and the other eight photobio-reactors with sterile WTF collected from a pond cultivation of opencast tilapia located at the Instituto Tecnológico de Boca del Rio. These were previously filtered through a 100 µm packed mesh column for phytoplankton with cotton and polyester fiber. The water was incorporated into the respective photobioreactors and disinfected using 0.8 ml l^{-1} of NaClO and neutralized with 0.75 g l^{-1} of $Na_2S_2O_3.5H_2O$ for 2 h under continuous aeration. The initial concentration of NO_2-N, NO_3-N, NH_3-N and PO_4^{-3} in WTF was determined (1, 8.4, 0.31 and 1.5 mg/l, respectively) using an HANNA Multiparameter (Model HI83099). Each culture medium was inoculated separately with 1.0 × 10^6 cells ml^{-1} of *C. vulgaris* and *N. oculata.* The volume of the inoculum to be used was determined by cell count in a Neubauer chamber (Pica-Granados et al., 2004) using the following equation:

$$V_2 = \frac{(C_1.V_1)}{C_2} \quad (1)$$

Where V_1= volume of reactor operation (6000 ml), C_1= initial cell density in the reactor (1.0 × 10^6 cells ml^{-1}), V_2= volume of inoculum required for the reactor (ml), and C_2= cell density of the inoculum at the time of inoculation of the reactor (cells ml^{-1}).

Duration of the culture was 10 days at 20 ± 2°C. Light intensity of 79.88 µmol m^{-2} s^{-1} with multi-LED lamps or white light was used. Cell density (cells ml^{-1}), lipid content (%), biomass productivity (g l^{-1} d^{-1}), lipid productivity (mg l^{-1} d^{-1}) and fatty acid quantification (%) were evaluated. All results were expressed as mean ± standard deviation. Statistical analysis was done using analysis of variance (ANOVA); $P<0.05$ was accepted as statistically significant. Stat Soft, Inc. (2004) STATISTICA V.7 was used for analysis. A 400 ml sample was used from treatment in the exponential (sixth day) and stationary (eighth day) phases based on growth kinetics. Samples were then filtered to gravimetrically determine dry and wet weight of the biomass. Once dried, samples were taken. We then proceeded to lipid extraction using the Soxhlet method with a mixture of chloroform/ methanol (1:2 v/v) (Halim et al., 2012). The product of the extraction was considered as the lipid content per species and treatment which, after being weighed, was stored in amber vials for later quantification of fatty acid profiles. For quantification of fatty acids, esterification or derivatization of the lipid fraction of the samples was carried out with the addition of 2.63 g of KOH, 30 ml of methanol and 10 ml of water. Evaporation was carried out and cooled to room temperature. Thirty ml of HCl was added to 3% in methanol and again evaporated to salt formation. The sample was rinsed with 20 ml of distilled water and placed in a separation funnel to which 30 ml of hexane was added. The sample was stirred for 1 min and then left to decant to obtain two phases and to scrape the precipitate. The supernatant was heated (40°C) to evaporate the solvent residues and subsequently diluted to 200 µl. Samples were finally stored in amber vials. The supernatant was heated (40°C) to evaporate the residue of the solvent and subsequently gauged to 200 µl to finally keep the samples in amber vials (Lepage and Roy, 1984). Fatty acids were identified and quantified with a gas chromatograph (Perkin Elmer, Model Autosystem, Flame ionization)

Table 1. Cell density average for *Nannochloris oculata* and *Chlorella vulgaris* according to the different treatments.

Microalgae strains	Light conditions	Basal bold's medium	Wastewater tilapia farming
		Cell density (cells ml^{-1})	
Nannochloris oculata	Multi-LED	$2.52 \times 10^7 \pm 1.12 \times 10^7$	$4.75 \times 10^7 \pm 9.63 \times 10^6$
	White light	$6.27 \times 10^7 \pm 1.02 \times 10^7$	$4.52 \times 10^7 \pm 1.17 \times 10^7$
Chlorella vulgaris	Multi-LED	$8.83 \times 10^7 \pm 1.25 \times 10^7$	$5.63 \times 10^7 \pm 1.06 \times 10^7$
	White light	$6.54 \times 10^7 \pm 1.04 \times 10^7$	$3.43 \times 10^7 \pm 9.91 \times 10^6$

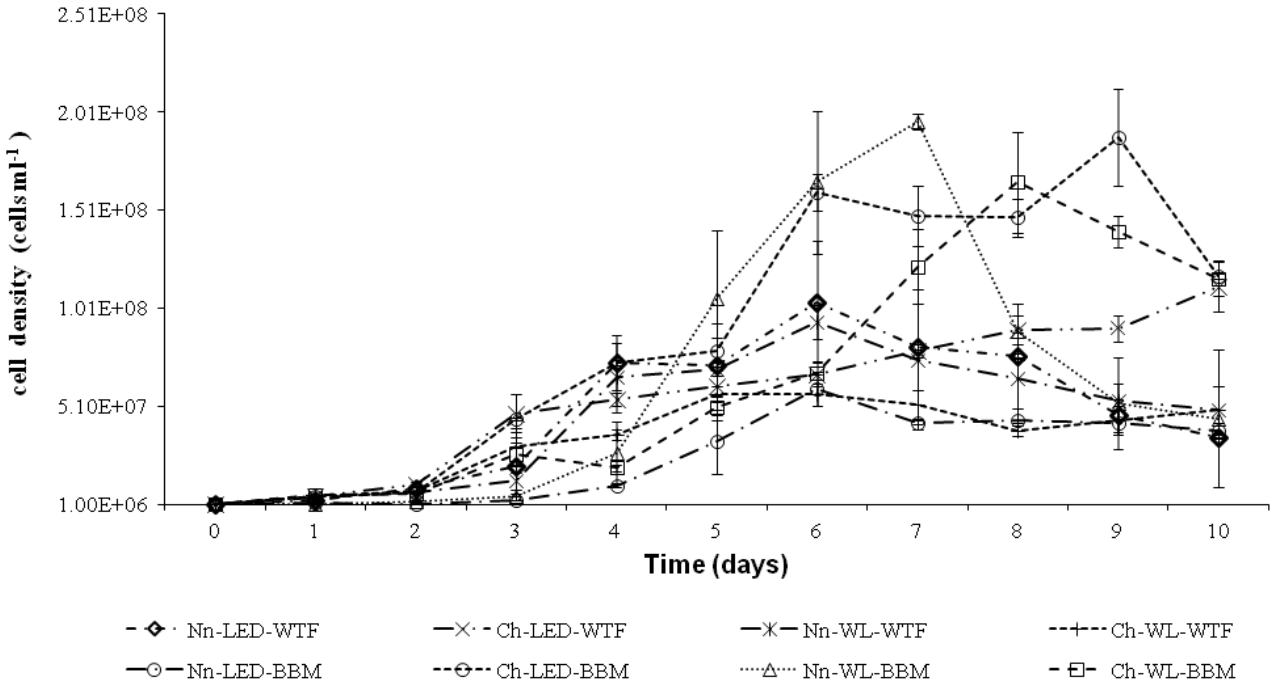

Figure 2. Comparison of cell density of *Nannochloris oculata* (Nn) and *Chlorella vulgaris* (Ch) in Wastewater Tilapia Farming (WTF) and Bold's Basal Medium (BBM) in multi-LED lighting (LED) and white lights (WL).

with an INNOWax capillary column (30 m in length × 0.320 mm in diameter). Nitrogen (N_2) was used as carrier gas. The injector temperature was 250°C and the detector was 300°C. Oven temperature was 150°C (4 min) with a ramp of 5°C min^{-1} to 190°C with a ramp of 2°C min^{-1} to 250°C (11 min). The injection volume was 2 µl per sample.

RESULTS

The results of this study indicate that, in general, the cultivations had a continuous and efficient growth in all tested treatments. It was observed that type of micro-algae and type of culture medium as well as all the interactions among the effects were significant ($P<0.05$). However, the type of lighting had no effect on cell growth. Except for treatment where the microalga *N. oculata* was under BBM white light illumination, most of the microalgae in the different treatments reached the exponential phase between the fifth and sixth day, whereas the first required 7 days and attained a cell density significantly ($P = 0.00004$) higher than that achieved by other algae (1.96×10^8 cells ml^{-1}) under study (Figure 2). The most efficient average cell density was presented during cultivation of *C. vulgaris* in BBM with multi-LED lighting (8.83×10^7 cells ml^{-1}), whereas the less efficient average cell density is presented in the same species in the WTF with white light. The highest average density of *N. oculata* was obtained in BBM with white light illumination (6.27×10^7 cells ml^{-1}), significantly higher than that achieved with the same microalgae but with WTF under the same lighting conditions (Table 1). The total biomass productivity of *C. vulgaris* that occurred during the study regardless of the treatment was in a range of 0.031-0.085 g l^{-1} d^{-1}, whereas for *N. oculata* it was 0.014-0.057 g l^{-1} d^{-1}, presenting significant differences between types of microalgae (Table 2). This

Table 2. Percent lipids, productivity of biomass and lipids of *Nannochloris oculata* and *Chlorella vulgaris* in WTF and BBM under two lighting conditions.

Microalgae strains	Culture media	Light conditions	Growth phase	Lipids (%)	Biomass productivity (g l^{-1} d^{-1})	Lipids productivity (mg l^{-1} d^{-1})
Nannochloris oculata	Bold's basal medium	Multi-LED	Exponential	57.80 ± 10.14	0.0141 ± 0.0002	8.1786 ± 5.7579
			Stationary	28.90 ± 5.13	0.0313 ± 0.0004	9.0313 ± 1.5026
		White light	Exponential	6.21 ± 0.19	0.0357 ± 0.0115	2.2083 ± 0.6482
			Stationary	10.21 ± 0.23	0.0571 ± 0.0074	5.8438 ± 0.8839
	Wastewater of tilapia farming	Multi-LED	Exponential	51.57 ± 7.22	0.0205 ± 0.0025	10.6071 ± 0.2020
			Stationary	33.34 ± 0.34	0.0279 ± 0.0088	7.1875 ± 3.0273
		White light	Exponential	42.49 ± 11.73	0.0219 ± 0.0009	9.2500 ± 2.1802
			Stationary	25.43 ± 3.36	0.0320 ± 0.0047	7.9688 ± 0.1105
Chlorella vulgaris	Bold's basal medium	Multi-LED	Exponential	27.66 ± 5.72	0.0459 ± 0.0028	12.6250 ± 1.8435
			Stationary	21.04 ± 4.99	0.0854 ± 0.0055	17.8281 ± 3.1157
		White light	Exponential	34.94 ± 2.36	0.0384 ± 0.0015	13.3958 ± 0.3830
			Stationary	22.41 ± 2.18	0.0543 ± 0.0132	11.3750 ± 3.6681
	Wastewater of tilapia farming	Multi-LED	Exponential	34.75 ± 2.31	0.0316 ± 0.0033	10.6429 ± 0.4293
			Stationary	27.03 ± 4.99	0.0456 ± 0.0061	12.5000 ± 2.1802
		White light	Exponential	39.38 ± 6.63	0.0446 ± 0.0080	17.2917 ± 0.1768
			Stationary	34.96 ± 4.94	0.0401 ± 0.0021	13.3958 ± 0.3830

may be due to the fact that microalgal biomass productivity is directly dependent on the species studied and on culture conditions (Chojnacka and Marquez-Rocha, 2004; Simionato et al., 2013). On the other hand, the results show that the highest lipid content was reached with *N. oculata* under multi-LED lighting in BBM and WTF (58 and 52%, respectively) in the exponential growth phase. Moreover, lipid productivity for both species was variable, according to medium as well as to type of lighting, reaching 2-18 mg l^{-1} d^{-1} productivities. *C. vulgaris* presented the highest productivity for both culture media (BBM and WTF). However, for *C. vulgaris* in BBM with multi-LED lighting, the highest productivity was in the stationary phase (17.83 mg l^{-1} d^{-1}) unlike in WTF with white light as lighting was in the exponential phase (17.30 mg l^{-1} d^{-1}). Lipid productivity depends mainly on the type of microalgae, type of culture medium, lighting conditions and growth phase. Lipid content was similar to that achieved by *C. vulgaris* in WTF with white lighting of 37% with an average biomass productivity of 0.04 g l^{-1} d^{-1}. For cultivations in WTF as a stress condition for its limitation of nitrogen compared with BBM, results show that for both microalgae, the percentage and lipid productivity for WTF were higher (25-42 %) than in MBB (6-35%), mainly in those treatments using white light illumination (Table 2). In addition, *N. oculata* and *C. vulgaris* showed the highest concentration of mono and polyunsaturated oleic, linoleic, palmitic, stearic, eicosanoic, arachidonic and eicosapentaenoic fatty acids in their lipid composition with fractions of 2-43% and up to 75% total (Table 3).

Table 4 shows the results of the removal efficiencies average of ammonium, nitrites, nitrates and phosphates in the WTF by type of microalgae and lighting. The microalgae *C. vulgaris* in white light presented the greater removal efficiency of nitrogen compounds. The removal efficiency of nitrite with 83% reported highest efficiency, followed by nitrates and ammonium (52 and 23%, respectively). However, higher removal efficiency of phosphates with 66% was in Multi - LEDs. This result is consistent to what was reported for low densities of inoculum (1×10^6 cells ml^{-1}) where the ranks ranged from 63 to 73% (Lau et al., 1995; Jiménez del Río, 1996; Neori et al., 2004; Hanumantha-Rao et al., 2010).

On the other hand, in terms of energy consumption, the treatment with multi-LEDs had an approximate consumption of 79.88 µmol m^{-2} s^{-1} (light intensity), which would amount to 18.94 W m^{-2}, taking as summarized data of equivalence to 1800 µmol m^{-2} s^{-1} ≡ 427 W m^{-2} (Gal et al., 1999).

Comparing energy consumption by type of lighting, white lights was higher than multi-LEDs (40 and 18.94 W, respectively). The photobioreactors operated for 10 days, reached an approximate consumption of 9.6 and 4.32 kWh for white light and multi-LEDs, respectively. The biomass productivity (Table 2) for *C. vulgaris* in both BBM and WTF, stationary phase, multi-LED was of 0.0854 and 0.0456 g l^{-1} d^{-1}, respectively. Therefore, the biomass productivity in 10 day of operation of the photobioreactors (6 l by reactor) was of 5.12 and 2.74 g for BBM and

Table 3. Percent composition (%) of fatty acids in the lipid fraction extracted from *Nannochloris oculata* and *Chlorella vulgaris* grown in photobioreactors in WTF and BBM under two lighting conditions (multi-LED and white light).

Fatty acid	Fatty acid	Treatments							
		Nn[e]-LED[a]-WTF[C]	Nn-LED-BBM[d]	Nn-WL[b]-WTF	Nn-WL-BBM	Ch[f]-LED-WTF	Ch-LED-BBM	Ch- WL - WTF	Ch- WL-BBM
		(%)							
Lauric acid	C12:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Myristic acid	C14:0	0.67	0.00	0.00	0.00	0.00	0.06	0.00	0.00
Palmitic acid	C16:0	11.66	1.58	12.00	9.41	8.32	6.10	1.91	7.29
Stearic acid	C18:0	6.06	1.54	16.97	8.55	19.65	2.08	10.90	3.32
Oleic acid	C18:1	13.68	6.40	18.37	9.25	14.81	13.05	7.06	7.23
Linoleic acid	C18:2	12.15	7.98	12.83	10.26	4.55	1.92	1.44	3.78
Linolenic acid	C18:3	6.97	2.81	0.00	2.59	6.46	3.21	3.64	2.55
Eicosanoic acid	C20:1	12.09	6.11	5.65	30.77	16.01	8.35	8.44	10.24
Arachidonic acid	C20:4	1.26	0.00	3.18	0.00	3.83	7.59	15.09	12.24
	Total	64.54	26.42	69.00	70.83	73.63	42.36	48.48	46.65

[a]Multi-LED lighting; [b]White light; [c]wastewater of tilapia farming; [d]bold's basal medium; [e]*Nannochloris oculata*; [f]*Chlorella vulgaris*.

Table 4. Removal efficiencies average of nitrogen compounds and phosphate in *Chlorella vulgaris* and *Nannochloris oculata* cultured in WTF for both types of lighting.

Treatment			Removal efficiency (%)			
			Ammonium	Nitrite	Nitrate	Phosphate
			(NH_3-N)	(NO_2-N)	(NO_3-N)	(PO_4^{-3})
WTF	Multi-LEDs	*Chlorella vulgaris*	1.32 ± 0.26	30 ± 4.56	41.73 ± 1.56	66.33 ± 6.28
	White light	*Chlorella vulgaris*	22.60 ± 4.12	83 ± 3.49	51.95 ± 2.47	45.00 ± 3.19
	Multi-LEDs	*Nannochloris oculata*	12.50 ± 2.31	0.00 ± 0.00	0.00 ± 0.00	24.96 ± 5.16
	White light	*Nannochloris oculata*	12.50 ± 1.89	0.00 ± 0.00	3.65 ± 0.15	47.48 ± 2.91

WTF, respectively; with energy consumption per gram of 0.84 and 1.6 kWh g^{-1} in BBM and WTF, respectively. The greatest energy consumption per gram of biomass for *N. oculata*, was in white light in BBM (2.80 kW g^{-1}). Comparing the best results among type of lighting in BBM, the requirement of energy per gram of biomass was greater with white light in *N. oculata* (2.80 kWh g^{-1}). However, comparing by type of lighting in WTF, *N. oculata* presented greater energy consumption per gram in white light than *C. vulgaris* in multi-LEDS (2.80 and 1.6 kW g^{-1}, respectively).

DISCUSSION

In this study, microalgae with the highest average density was *C. vulgaris* in BBM with 88.3 × 10^6 cells ml^{-1} during 10 days of cultivation, much higher than the densities obtained in the above-mentioned studies. It is important to note that there were higher densities in all of the treatments reported under different lighting conditions (Figure 2).

De-Bashan et al. (2002) conducted studies on cultures of *C. vulgaris*, *Azopirillum brasilense* and *C. vulgaris/A. brasilense*, showed that the highest density was in discontinuous cultivations with 4 × 10^6 cells ml^{-1}. Cleber et al. (2008) studied chlorophyll content and profile of minerals in the microalga *C. vulgaris* grown in hydroponic wastewater solution. Cultivations were carried out in BBM as control, concentrations of 100% hydroponic residual solution (HRS), 50% residual hydroponic solution and 50% deionized water (HRS50), 25% of residual hydroponic solution and 75% of deionized water (HRS25). The cultivation period was 7 days, obtaining average densities at the end of the period of 10.6 × 10^6, 5.7 × 10^6, 4.2 × 10^6 and 10.1 × 10^6 cells ml^{-1} in BBM, HRS, HRS50 and HRS25, respectively. Furthermore, recent studies to determine the effect that produces leachates of the biosolids on freshwater biota demonstrated an effect on *N. oculata* affecting their growth at concentrations >500 ppm of leachate after being cultivated with 12 h light at 31.5 µmol m^{-2} s^{-1} and 12 h dark for 14 days at concentrations of 50, 200, 500 and 1000 ppm (Flores et al., 2010). Results indicate that the highest and lowest cell density reached 50 and 500 ppm with 1.77 × 10^6 and 0.65 × 10^6 cells ml^{-1}, respectively, con-

cluding that the leachate can function as fertilizer for the growth of *N. oculata* to concentrations not higher than 50 ppm.

As shown in previous studies, various types of waste are used for the cultivation of *C. vulgaris* and *N. oculata* as culture media. Average cell density at the end of the culture for periods between 7 and 12 days with an initial inoculum of 1.0×10^6 cells ml^{-1} was 2.5×10^6 to 10.6×10^6 cells ml^{-1} and 0.65×10^6 to 1.77×10^6 cells ml^{-1} for *C. vulgaris* and *N. oculata*, respectively.

Mata et al. (2010) remark that, under nitrogen limitation, *C. vulgaris* and *N. oculata* maintained a production of 0.02-0.20 and 0.37-0.48 g l^{-1} d^{-1}, respectively. In addition, the stress situation during cultivation caused the microalgae to generate a higher content of lipids (from 75%) in comparison to what is expected (20-50%), with lower biomass productivity (0.02 g l^{-1} d^{-1}) in relation to what is expected (0.40 g l^{-1} d^{-1}). In another study, Chiu et al. (2008) reported that in semicontinuous cultures of *Chlorella* sp. with low and high cell density for CO_2 reduction, biomass productivity of 0.037-0.053 g l^{-1} d^{-1} was obtained; values similar to that obtained in this study for both strains under all culture conditions.

Furthermore, Liang et al. (2009) reported biomass productivity of 0.010-0.254 g l^{-1} d^{-1} in culture of *C. vulgaris* under growth conditions of heterotrophs, autotrophs and mixotrophs with a maximal lipid content of 38% under autotrophic conditions. These results, indicate that under conditions of stress due to a low concentration of nitrogen (primarily nitrates), there is an increase in lipid content in biomass at a range of 15 to 58% (dry weight) depending on the species of microalgae (Mata et al., 2010; Probir et al., 2011; Kirrolia et al., 2013). This behavior was previously observed in other studies (Mata et al., 2010; Kirrolia et al., 2013). Therefore, biomass and lipid productivity can be increased up to 60% under culture conditions with nitrogen deficiency and high concentrations of CO_2 (Chiu et al., 2009; Gouveia and Oliveira, 2009; Liang et al., 2009; Rodolfi et al., 2009; Mata et al., 2010; Borges et al., 2011; Kirrolia et al., 2013).

On the other hand, Guerrero-Cabrera et al. (2014) cultivated three species of microalgae (*Monoraphidium* SP., *Chlorella* SP. and *Scenedesmus* SP.) in three volumes of WTF (1.5, 4 and 9 l, respectively) as culture medium and BBM, and compared the specific rate of growth, volumetric productivity (g l^{-1} d^{-1}), biomass productivity (g l^{-1}), as well as protein and lipid content. They reported that *Scenedesmus sp*. in BBM; it produced a higher specific speed of growth and volumetric productivity (0.332 g l^{-1} d^{-1}) than WTF in 1.5 L of volume. Also reported was *Chlorella sp*, which also showed a higher lipids volumetric productivity (0.011 g L^{-1} d^{-1}) for BBM to WTF to 1.5 L of volume. The maximum lipids concentration in percentage was for *Monoraphidium* SP. in WTF, 1.5 L with 17.8%.

Due to its rapid speed of growth and mainly due to its high content of lipids with a rich fraction of saturated and monosaturated fatty acids, preferably C16–C20 polyunsaturated chain, numerous strains of microalgae have been studied as a potential source of triacylglycerides (TAG), the main raw material for biodiesel production (Delrue et al., 2012; Hoekman et al., 2012; Lohrey et al., 2012; Chen et al., 2013; Wei et al., 2013; Singh et al., 2014; Taher et al., 2014).

Some studies have shown that the highest content of lipids present in *N. oculata* and *C. vulgaris* was achieved in nitrogen deficient cultures with a higher content of mono and polyunsaturated fatty acids (Rodolfi et al., 2009; Mata et al., 2010; Kirrolia et al., 2013; Singh et al., 2014; Taher et al., 2014). Therefore, cultures of *N. oculata* illuminated with multi-LEDs and in WTF may be an attractive and economic alternative for the generation of biodiesel due to the high percentage of lipids and mono and polyunsaturated fatty acids.

Conclusion

The highest biomass productivity and highest cell density was in *C. vulgaris* in BBM and multi-LEDs. *N. oculata* in multi-LEDs reached the highest percentages of lipids in both media. The lipid productivity for both species was variable both for culture medium and lighting condition. Fatty acid composition in the different treatments was mainly saturated, where the highest percentages were in WTF. Based on the results, it is concluded that *N. oculata* and/or *C. vulgaris* in WTF in multi-LEDs are an economic and sustainable alternative in a scheme of cultivation of microalgae with the greatest potential as a generator of biodiesel.

Conflict of interests

The authors did not declare any conflict of interests.

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