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EFFECT OF LIGHT INTENSITY ON REMOVAL OF CONTAMINANTS IN TILAPIA RESIDUAL EFFLUENTS WITH MOCROALGAE

Efecto de la intensidad de luz sobre el crecimiento y remoción de contaminantes en efluente residual de tilapia con microalgas

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Key words: chlorophytes, biomass productivity, aquaculture contaminants, light intensity.

ABSTRACT

Residual aquaculture effluents are discarded into the environment, causing adverse effects on water bodies through eutrophication. Therefore, looking for a treatment that extracts the contaminants and adds benefits by cultivating microalgae in the effluents is necessary. In this research, *Chlorella vulgaris* and *Nannochloropsis oculata* were cultivated in aquaculture waste effluent using two lighting conditions (40.5 and 72.9 μmol m^{-2} s^{-1}). The results show that the time the exponential and stationary phases are reached was not influenced by light, but cell growth, production, and biomass productivity were. The best results were for the condition of 72.9 μmol m^{-2} s^{-1} in stationary phase ($4.62\times10^7 \pm 2.12\times10^5$ and $4.45\times10^7 \pm 2.33\times10^6$ cell mL^{-1}; 0.684±0.124 and 0.718±0.122 g L^{-1}; 0.043 ± 0.008 and 0.048 ±0.008 g L^{-1} d^{-1}) with C. vulgaris and N. *oculata*, respectively. Cultures at 72.9 μmol m^{-2} s^{-1} and the stationary phase were better for removing nitrates, phosphates, and COD (80-97%, 25-50%, and 43-89%) in microalgae and growth phases. While for ammonium and nitrites, the highest removal efficiency was obtained with 40.5 μmol m^{-2} s^{-1} in the stationary phase. Therefore, light intensity is an essential factor to consider when there are high concentrations of contaminants On the other hand, the light intensity of 72.9 μmol m^{-2} s^{-1} was also the most suitable for the highest biomass production and productivity in the stationary phase.

Palabras clave: clorofitas, productividad de biomasa, contaminantes acuícolas, intensidad de luz.

RESUMEN

Los efluentes residuales acuícolas son desechados al ambiente causando efectos adversos en los cuerpos de agua a través de la eutrofización. Por ello, es necesario buscar un tratamiento con el cual extraer los contaminantes y agregar un beneficio a través del cultivo de microalga en los efluentes. En esta investigación se realizó el cultivo de *Chlorella vulgaris* y *Nannochloropsis oculata* en efluente residual acuícola utilizando dos condiciones de iluminación (40.5 y 72.9 µmol m^{-2} s^{-1}). Los resultados obtenidos muestran que el tiempo en el que se alcanzan las fases exponencial y estacionaria no se vio influenciado por la luz, pero sí el crecimiento celular, producción y productividad de biomasa. Las mejores resultado fueron para la condición de 72.9 µmol m^{-2} s^{-1} en fase estacionaria ($4.62\times10^7 \pm 2.12\times10^5$ y $4.45\times10^7 \pm 2.33\times10^6$ cel mL^{-1}; 0.684±0.124 y 0.718±0.122 g L^{-1}; 0.043 ±0.008 y 0.048 ±0.008 g L^{-1} d^{-1}) con *C. vulgaris* y *N. oculata*, respectivamente. Los cultivos a 72.9 µmol m^{-2} s^{-1} y la fase estacionaria. Por tanto, la intensidad de la luz es un factor importante a considerar cuando hay altas concentraciones de contaminantes. Por otro lado, la intensidad lumínica de 72.9 µmol m^{-2} s^{-1} también fue la más adecuada para la mayor producción y productividad de biomasa en la fase estacionaria.

INTRODUCTION

Residual aquaculture effluents (RAE), as a result of the intensification of aquaculture, need to be disposed of more sustainably, saving resources in their processing and, if possible, obtaining high-value compounds with the recycling of nutrients and by-products (Campanati et al. 2022). RAEs have a similar composition, although there are differences in the quality and quantity of components depending on the location, the species cultivated, and the cultivation practices adopted. In general, they contain uneaten food and feces that can represent 30 percent of uneaten dry food and 30 percent of consumed food that is ingested as feces (Chatla et al. 2020); in addition to solids from external sources, the growth of microalgae and bacteria (Chadwick et al. 2014). The farming systems that produce the most effluents are semi-intensive and intensive since they use more inputs. Therefore, they create more solid waste and nutrients that probably cause acute toxic effects and long-term environmental risks (Chatla et al. 2020), such as eutrophication (Martínez-Cordero et al. 2021; Hernández-Pérez & Labbé, 2014), hypoxic events and water acidification (Campanati et al. 2022). For this reason, avoiding and mitigating the introduction of harmful effluents into the environment should be vital in developing intensive industrial aquaculture to minimize the impacts of pollution (Li et al. 2020). According to the data provided by the UN (2022), the population will increase to 9.7 billion by 2050; therefore, in addition to intensifying crops and demanding more water, the same population growth and climate change cause increasing water scarcity. (FAO 2021), space and food (Campanati et al. 2022). In 2019, the UN launched a global campaign on sustainable nitrogen management and set the goal of halving waste by 2030; it is also about increasing treatment systems and increasing water recovery in the event of moving to a more circular economy (Sealy, 2021), in Mexico NOM-001-SEMARNAT-2021 (SEMARNAT 2022) has been established, which establishes the maximum permissible limit of total N towards rivers, streams and canals of 25, 30 and 35 mg L^{-1} and total P of 15, 18 and 21 mg L^{-1}, respectively.

Some of the treatments used in aquaculture are removal of solids by sedimentation and mechanical filtration, extractive treatment with microalgae (Chadwick et al. 2014), wetlands (Lin et al. 2002; Hu et al. 2017), some techniques are approached from discharges during cleaning and harvesting through vegetated infiltration areas or crop irrigation (Yeo et al. 2004), while other potential pollution mitigation methods, which present high initial investment costs and energy consumption, are: effective indoor recirculating aquaculture systems (RAS) and laboratory culture; integrated multitrophic aquaculture (IMTA), which incorporates species of different trophic or nutritional levels; the biofloc technique, which involves manipulation of the C/ N ratio to convert toxic nitrogenous waste into beneficial microbial protein (Chatla et al. 2020). One of the treatments that is gaining strength is the extractive treatment with algae since these use solar energy to convert the nutrients present in the effluents into valuable resources through photosynthesis. Specifically, the nutrients produced in aquaculture effluents are solid

(carbon, nitrogen, and phosphorous), soluble (carbon dioxide, ammonia, orthophosphate, and trace elements (Chadwick et al. 2014). Nutrients that microalgae can recycle and favor the formation of some compounds, as observed with *Nannochloris maculata*, produce more lipids in shrimp effluents than in conventional medium (Conway) (Campanati et al. 2022). The same behavior has been observed in the removal of contaminants such as copper (Martínez-Macias et al. 2019; Aguilar-Ruiz et al. 2020), so the biomass produced can be directed to other industries for the extraction of high-value compounds or biofuels (Campanati et al. 2022). The factors to consider when using microalgae for bioremediation of effluents are nutrient concentration, light intensity, temperature, and turbulence, considering that nutrient uptake will depend on available light energy (Richmond, 2004). For this reason, this research aims to observe the effect of two light intensities on The growth of contaminants and removal of contaminants (nutrients) in tilapia residual effluents with two species of chlorophyte microalgae such as *N. oculata* and *C. vulgaris*.

MATERIALS AND METHODS

Aquaculture effluent

In the fattening stage, effluents from two tilapia aquaculture ponds were manually collected in 20 L plastic containers. The first pond with *Oreochromis niloticus* tilapia (ET1) and the second with *O. niloticus* rocky mountain var., white (ET2), both under greenhouse conditions located at the Boca del Río Technological Institute, in the municipality of Boca del Río in the state of Veracruz. The effluent was settled for 24 hours and filtered with polyester cloth on the upper part, filter paper, and 100 µm mesh on the bottom. After filtering, the effluents were chemically sterilized using 1 mL of commercial chlorine per liter of effluent. For neutralization, 0.5 grams of sodium thiosulfate ($Na_2S_2O_3.5H_2O$) per liter of effluent was used, maintaining constant aeration for 24 h (ETE). The effluent was characterized before and after sterilization, determining the initial concentration of NO_3-N, NO_2-N, NH_3-N, PO_4^{-3}, and COD using a HANNA multiparameter (Model HI83099).

Culture conditions

Strains of *C. vulgaris* and *N. oculata* obtained from the Ensenada Center for Scientific Research and Higher Education (CISECE) and the University of Texas (UTEX) collection were used, respectively. The strains were maintained under controlled conditions using Bold's Basal Medium (MBB) (Stein, 1979), and 200 mL Erlenmeyer flasks (Ferrer-Álvarez et al. 2015) at 22 ±2.0 °C, with a photoperiod of 24 hours and a light intensity of 40.5 µmol. m^{-2} s^{-1}, a luxometer (digital luminosity meter) STEREN HER-408 was used. For scaling to photobioreactors, four 500 mL and four 1000 mL Erlenmeyer flasks were used for 7 days; the seeding density was 1×10^6 cells mL^{-1}, and the amount of inoculum was calculated with Eq.1 at a temperature of 22 ±2.0 °C, photoperiod of 24 hours at 40.5 µmol m^{-2} s^{-1} and constant aeration (**Fig. 1**).

$$V_2 = (C_1V_1)/C_2 \quad \text{Eq.(1)}$$

Where: V_1: Operating volume in the reactor (500 ml), C_1: Initial cell density in the reactor (1×106 cells mL^{-1}), V_2: Volume of inoculum required for the reactor (ml), C_2: Cell density of the inoculum at the time of inoculating the reactor (cells mL^{-1}).

Growth phases

The determination of the exponential (EXP) and stationary (STA) growth phases of C. vulgaris and *N. oculata* was carried out in 1000 mL flasks in MBB and ETE (with the effluent with the highest nutrient content); the seeding density was 1×10^6 cells mL^{-1}, the cell count was performed using a Neubauer chamber by the method of Pica-Granados et al. (2004), for the calculation of the inoculum, Eq. 1 was used where V_1 = 1000 mL. The growth conditions were two light intensities (40.5 and 72.9 µmol m^{-2} s^{-1}) at 22 ± 1.0 °C, with a photoperiod of 24 light hours and constant aeration.

Photobioreactors

The photobioreactors (experimental units) were filled with 6 L of previously characterized and sterilized tilapia culture effluent. After inoculating with 1×10^6 cells mL^{-1}, the inoculum was taken from the continuous cultures previously prepared with MBB in 1000 mL flasks in the exponential phase. The volume of the inoculum was determined by cell count using the equation Eq.1, where V_1= 6000 mL. The growth conditions were two light intensities (40.5 and 72.9 µmol m^{-2} s^{-1}) at 22 ± 1.0 °C, with a photoperiod of 24 light hours and constant aeration (**Fig. 2**).

Removal efficiency.

To determine the removal efficiency of nitrogenous compounds, phosphates, and organic material, 50 ml

Fig. 1. Maintenance and propagation of strains.

Nannochloropsis oculata *Chorella vulgaris*

Fig. 2. Culture of microalgae in 6 L photobioreactors.

samples were taken every third day to evaluate the amount of NO_2-N, NO_3-N, NH_3-N, PO_4^{-3}, and COD removed from the effluent of tilapia by microalgae. The sample was centrifuged at 3500 revs for 15 min, using the supernatant for analysis. For the measurement of ammonium, nitrite, phosphate, and nitrate, the HANNA HI83099 multiparameter was used, previously calibrated with deionized water. For COD, the samples were digested in a HANNA brand HI 839800 COD reactor; at the end of the digestion, the sample was read in the HANNA HI83099 multiparameter. The pH was measured with a Science MED BM-25CW potentiometer (MICROPROCESSOR pH/mV METER).

The removal efficiency (η) expressed in % was determined with the equation Eq.2:

$$\eta = \frac{Ic - Fc}{Ic} x100 \qquad \text{Eq.(2)}$$

Where: Ŋ: Removal efficiency (%), Ic: Initial concentration (day 0) of the compound, Fc: Final concentration (every third day of culture).

Experimental design

The experiment consisted of 2^4 factorial analyses for cell density. Four factors were evaluated: A) Type of microalgae, B) Light intensity, C) Growth phase, and D) Culture medium in triplicate. For the efficiency of removal, production, and productivity in cultures with residual effluent (ETE with higher nutrient content), the factorial design was 2^3 where the factors evaluated are: A) Type of microalgae, B) Light intensity, and C) Phase of growth, triplicate. To determine production and productivity, 800 mL samples of the culture were collected in the exponential and stationary growth phases, which were kept refrigerated at 4 °C for sedimentation; the sedimented biomass was centrifuged in CRM GLOVE CENTRIFICIENT IV for 15 minutes at 3500 rev. Subsequently, the sample was filtered through a Whatman brand cellulose membrane of 110 mm diameter and dried to constant weight. Equations Eq, 3 and Eq, 4 were used to determine biomass production on a dry basis (BS) in mg mL^{-1} and productivity, respectively.

$$BP = \frac{[Wdmm(mg) - Wdm(mg)]}{800\ ml} \qquad \text{Eq.(3)}$$

$$BPD = BP/CD \qquad \text{Eq.(4)}$$

Where: BP: biomass production (mg $L0^{-1}$), Wdmm: dry membrane weight with microalgae (mg), Wdm: dry membrane weight (mg), BPD: biomass productivity (g L^{-1} d^{-1}), CD: culture days (d).

Statistic analysis

The statistical analysis was carried out using the analysis of variance with a $p < 0.05$, which was accepted as statistically significant; the STATISTICA 7.0 program was used.

RESULTS AND DISCUSSION

Residual effluent

The concentration of NO_2-N, NO_3-N, NH_3-N, PO_4^{-3}, and COD of the two sampled effluents are shown in **Table I**, where the characteristics and variations in the concentration of effluent compounds are observed after the sterilization, where the amount of nitrates decreased by 74.56% (ETE1) and 29.36% (ETE2), with nitrite no change was observed in the filtered and sterilized effluent, with ammonium and phosphate the changes were minimal, with COD it was observed a decrease of 2.06% (ETE1) concerning ET1 and 14.15% (ETE2) concerning ET2. Chacon et al. (2004) mention that the content of phosphates ($P\text{-}PO_4^{-3}$), ammoniacal nitrogen ($N\text{-}NH_4^{+}$) and COD vary if the analyzes are carried out on the raw, filtered or sterilized effluent, being the highest in the raw effluent, in regarding phosphate, he found a decrease of 11.7% (filtered) and 30% (sterilized), with ammonium of 13.95% (filtered) and 69.77% (sterilized), with COD of 61.16% (filtered) and 42.25% (sterilized). Therefore, after the ETE2 sterilization process, it presented higher concentrations of contaminants, which is why it was the effluent used in the tests.

TABLE I. RAW AND STERILE EFFLUENT PARAMETERS.

Parameters	ET1	ETE1	ET2	ETE2
pH	6.9	7.0	6.3	6.7
Nitrate (mg L^{-1})	22.8	5.8	21.8	15.4
Nitrite (mg L^{-1})	0.3	0.3	0.05	0.05
Ammonium (mg L^{-1})	0.52	0.70	0.51	0.46
Phosphate (mg L^{-1})	45.0	45.6	19.7	18.3
COD (mg L^{-1})	340.5	333.5	583.0	500.5

ET1: *Oreochromis niloticus* effluent, ET2: *O. niloticus* rocky mountain var., blanca effluent, ETE: Sterile effluent.

Growth kinetics

The results obtained indicate that all the factors tested significantly influence ($p<0.05$) the cell growth of the microalgae *C. vulgaris* and *N. oculata*, as well as most of the combinations except for the combination Microalga*Cultivation medium, Microalga *Growth phase and Microalgae*Light intensity*Growth phase. It was observed that with a low light intensity (40.5 μmol m^{-2} s^{-1}), there is no difference in growth with both culture media (**Fig. 3a**), but the difference is noticeable when a higher light intensity is used (**Fig. 3b**). The exponential and stationary phase of *C. vulgaris* and *N. oculata* at 40.5 μmol m^{-2} s^{-1} was obtained on days 6-10 and 12-16, respectively (**Fig. 1a**). The exponential and stationary phase of *C. vulgaris* and *N. oculata* at 72.9 μmol m^{-2} s^{-1} was obtained on days 5-11 and 12-14, respectively (**Fig. 3b**), the highest densities were obtained with MBB, reaching 8.17×10^7 $\pm3.50\times10^6$ cells mL^{-1} and 1.05×10^8 $\pm6.36\times10^5$ cells mL^{-1} with C. vulgaris and N. oculata, respectively. Comparing the growth of both lighting conditions with effluent as a culture medium (**Fig. 3c**), higher growth is observed with the highest lighting condition (72.9 μmol m^{-2} s^{-1}) reaching densities of 4.62×10^7 $\pm2.12\times10^5$ cells mL^{-1} and 4.45×10^7 $\pm2.33\times10^6$ cells mL^{-1} (**Table II**) with *C. vulgaris* and *N. oculata*, respectively. The analysis of variance showed a significant difference, with p=0.00088 and F=16.578, in the combination of the four factors used. The condition that most influenced growth was light intensity, and it was significantly different with p=0.00502 and F=10.560, which indicates that at 72.9 μmol m^{-2} s^{-1}, there was a greater assimilation of nutrients; it was observed with the MBB (control). The results obtained here agree with what was mentioned by Richmond (2004), who mentions that the most critical factor in cell growth is light intensity, as well as an adequate cell density, since at low densities, there are lower yields than at densities high planting, in addition to this, nutrients and photoperiod must be considered, this explains the difference in the times in which the exponential and stationary phase is reached, as well as the highest cell concentration in different studies (Chacón et al. 2004, Ferrer-Álvarez et al. 2015, El-Sheekh et al. 2016, Kumaran et al. 2023), where the most extended crop cycle was 27 days using a 12:12-hour light: dark photoperiod (Chacón et al. 2004).

Removal efficiency

The dynamic behavior of removal of nitrogenous, phosphate, and organic matter compounds varied depending on the intensity of the light used. Therefore, some compounds were removed more than others. At 40.5 μmol m^{-2} s^{-1} no nitrate removal is observed, this remains constant throughout the culture (**Fig. 4a**). However, at 72.9 μmol m^{-2} s^{-1} a decreasing trend is observed from the first day (**Fig. 5a**). In the case of nitrite, a similar trend is observed with both lighting conditions, increasing in the first days of culture and decreasing after day 8 (**Fig. 4b** and **Fig. 5b**). As for ammonium, a decreasing trend is observed in the first 8 days with both lighting conditions (**Fig. 4c** and **Fig. 5c**), and then it increased to decrease in the stationary phase, except for the culture with *C. vulgaris* at 72.9 μmol m^{-2} s^{-1} that reached the initial concentration on day 16 of the culture (**Fig. 5c**). In the case of phosphate at 40.5 μmol m^{-2} s^{-1}, an increase is observed after a gradual decrease until reaching the initial level (**Fig. 6a**), at 72.9 μmol m^{-2} s^{-1} an increase is observed in the day 2 and then the trend is decreasing until day 16 (**Fig. 7a**). In the case of COD, with both lighting conditions a decreasing trend is observed in the first 6-8 days of the culture, then it stabilizes until the end of the culture with both microalgae (**Fig. 6b** and **Fig. 7b**). The pH was 6.5 ±0.4 at 40.5 μmol m^{-2} s^{-1} with both microalgae, while at 72.9 μmol m^{-2} s^{-1} the pH varied from 6.7 ±0.27 with *C. vulgaris* and 6.9 ±0.35 with *N. oculata*. In removing nitrate and COD with *C. vulgaris* and *N. oculata*, light intensity significantly influenced p=0.00000 (nitrate) and p=0.00335 (COD), finding removals more significant than 80% of nitrate in both growth phases, and greater than 60 % with COD at 72.9 μmol m^{-2} s^{-1}. In the case of nitrite and ammonium, a significant difference was found (p=0.00000, p=0.00002, respectively), reaching removals greater than 79 % in the stationary phase with the lowest light intensity (40.5 μmol m^{-2} s^{-1}). In the case of phosphate with a p=0.00000, greater removal was found in both growth phases with both microalgae at 72.9 μmol m^{-2} s^{-1}, reaching the highest percentage in the stationary phase with 49.9 % and 50.4 % for *C. vulgaris* and *N. oculata*, respectively.

What was obtained in this study agrees with what other authors reported, a greater intensity of light demands a greater assimilation of nutrients. Rendón-Castrillón et al. (2015) mention that the assimilation of inorganic nitrogen is strongly dependent on light, both the intensity of light and its quality since they can control the assimilation of NO_3^- through the regulation of the synthesis and activity of the enzyme nitrate reductase (NR). The results obtained by Garatachia-Vargas (2018) show that the type of reactor used also influences the removal of nitrogenous and phosphate compounds since he found more significant removal in flat photobioreactors than in tubular

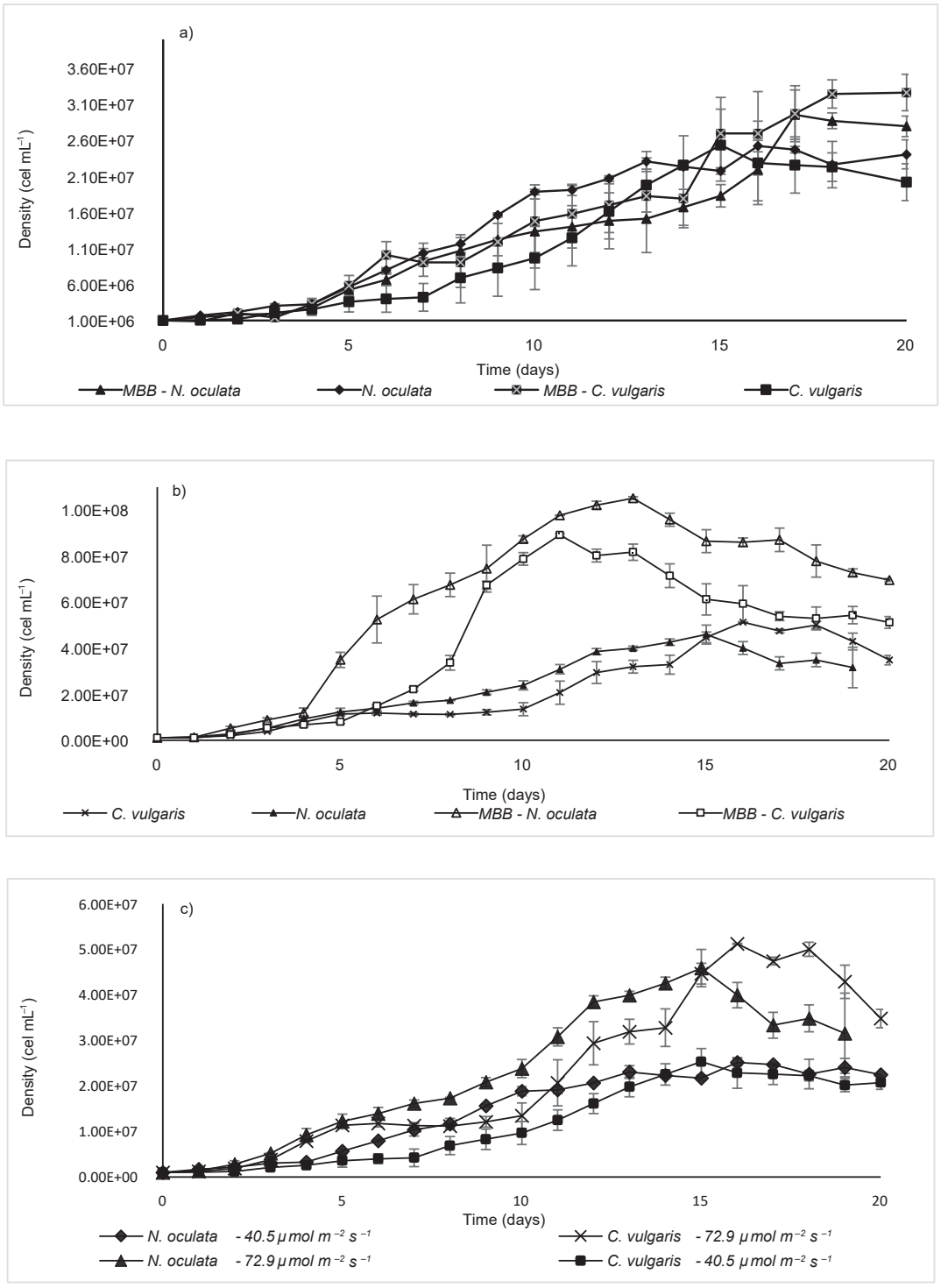

Fig. 3. Growth kinetics of *C. vulgaris* and *N. oculata*, a) 40.5 µmol m^{-2} s^{-1}, b) 72.9 µmol m^{-2} 650 $s^{-1}$and c) Effluent.

TABLE II. CELL DENSITY OF *C. vulgaris* AND *N. oculata* UNDER TWO LIGHTING CONDITIONS, CULTURE MEDIA, AND GROWTH PHASES.

Microalgae	Light intensity (μmol m^{-2} s^{-1})	Culture medium	EXP	Gowth phase		STA
			Cell density (cel mL^{-1})			
C. vulgaris	40.5	ETE2	9.68E+06	±2.53E+06	2.29E+07	±3.35E+06
		BBM	1.46E+07	±4.24E+05	1.95E+07	±1.20E+06
	72.9	ETE2	1.38E+07	±4.88E+06	4.62E+07	±2.12E+05
		BBM	7.88E+07	±2.76E+06	8.17E+07	±3.50E+06
N. oculata	40.5	ETE2	1.89E+07	±5.72E+05	2.31E+07	±8.06E+05
		BBM	1.04E+07	±5.83E+05	1.64E+07	±1.94E+06
	72.9	ETE2	2.39E+07	±3.46E+06	4.45E+07	±2.33E+06
		BBM	8.74E+07	±1.38E+06	1.05E+08	±6.36E+05

ETE2: O. *niloticus* rocky mountain var. Blanca sterile effluent; BBM: Bold's Basal Medium; EXP: Exponential; STA: Stationary.

photobioreactors using the same light intensity (110 μmol m^{-2} s^{-1}). In this study, it was observed that ammonium and nitrite were the compounds most assimilated at 40.5 μmol m^{-2} s^{-1}; this can be explained by the order in which the microalga uses nitrogenous compounds, where ammonium is absorbed first, then nitrites, and finally nitrate (Oscanoa-Huaynate et al. 2021, Medina-Aguilar 2016). However, there are cases in which nitrate, nitrite, and ammonium are assimilated simultaneously (Oscanoa-Huaynate et al. 2021). The decrease in ammonium has become a common characteristic in studies where dynamic behavior is shown (Ferrer-Álvarez et al. 2015, Paes et al. 2016, Garatachia-Vargas 2018); this is because the algae preferentially consume NH_4^+ instead of NO_3^- (Kube et al. 2018, Lin et al. 2016), because few biochemical steps and a low energy requirement are required for its assimilation (Lin et al. 2016), but when both forms are present (nitrate and nitrite), NO_3^- consumption is minimal until most of the NH_4^+ is removed (Kube et al. 2018). The low removal of nitrite at 72.9 μmol m^{-2} s^{-1} is explained because the higher the irradiance, the more nitrate is absorbed, which causes nitrite formation and is released due to its cytotoxic effects (Medina-Aguilar 2016). Similar effects have been observed with some diatoms and flagellates with sufficient nitrogen in response to rapid increases in irradiance. This behavior results from the reduction of nitrate to nitrite by nitrate reductase activity, not followed by further reduction to ammonia by nitrite reductase (Paes et al. 2016). In the case of phosphate, an increase was observed in the first days to 40.5 μmol m^{-2} s^{-1}, Ruiz-Martínez et al. (2015) observed in *Scenedesmus sp*. that the initial content of P (phosphorus) in the cell affects the consumption of P, since the higher the content of P in the internal biomass, the lower the rate of phosphate removal, the same observation obtained by Kube et al. (2018), who suggests increasing the concentration of P in the crop to increase the elimination rate and that was tested with Scenedesmus. Yao et al. (2011) observed with *S. quadricauda* that PO_4^{3-} was first adsorbed on the cell surface at a rapid rate and then slowly consumed by the cell, which is why they mention that in algae, there is a coexistence of intracellular and adsorbed phosphorus deposits, indicating that phosphate uptake by phytoplankton is a two-step kinetic process. In the case of surface-adsorbed P, it is in equilibrium with the surrounding wastewater and could be released after a long retention time, affecting domestic consumption. In this context, Kube et al. (2018) mention that to increase the consumption of P, the concentration of nitrogen must be increased since the limitation of this can decrease the thylakoid membrane, which governs photosynthesis (Beuckels et al. 2015). On the other hand, Grobbelaar (2004) mentions that when phosphorus limits growth, there is an excretion of alkaline phosphatases that mobilize the adsorbed organic P and make it available to the algae, which may explain the increase in phosphorus in the culture medium. Other factors that can influence the uptake of phosphates by algae are the absence of potassium, magnesium, or sodium and a high or low pH (Palacios-Sánchez 2022). The concept of starving algal cells before exposing them to wastewater has been raised to increase phosphorus removal rates. However, Kube et al. (2018) mention that this

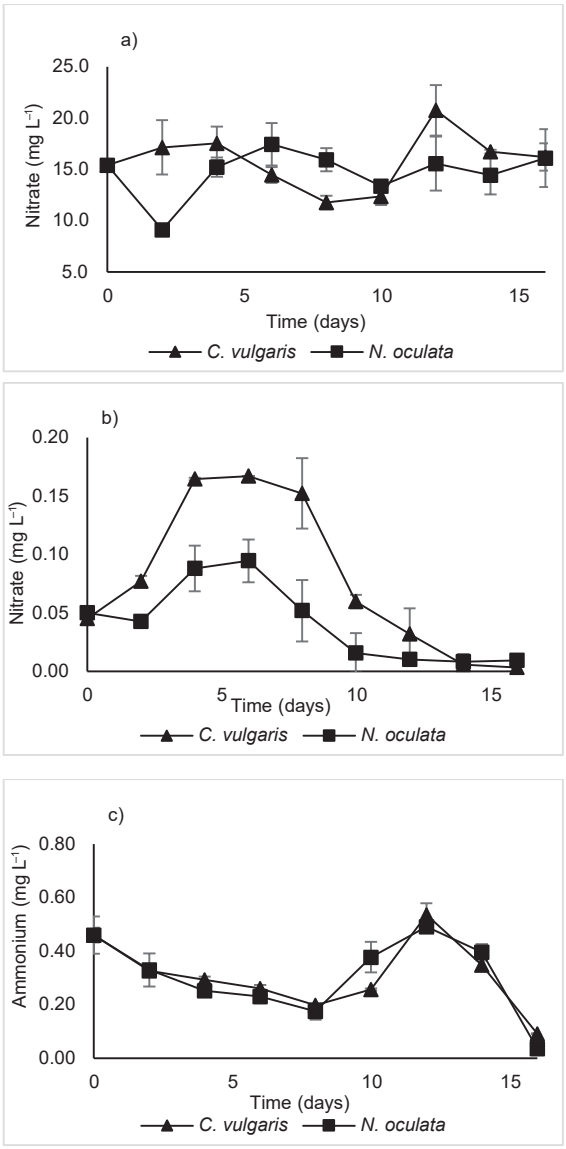

Fig. 4. Dynamic behavior of the concentration of a) nitrate, b) nitrite, c) ammonium with *C.vulgaris* and *N. oclata* grown in effluent at 40.5 mol m^{-2} s^{-1}.

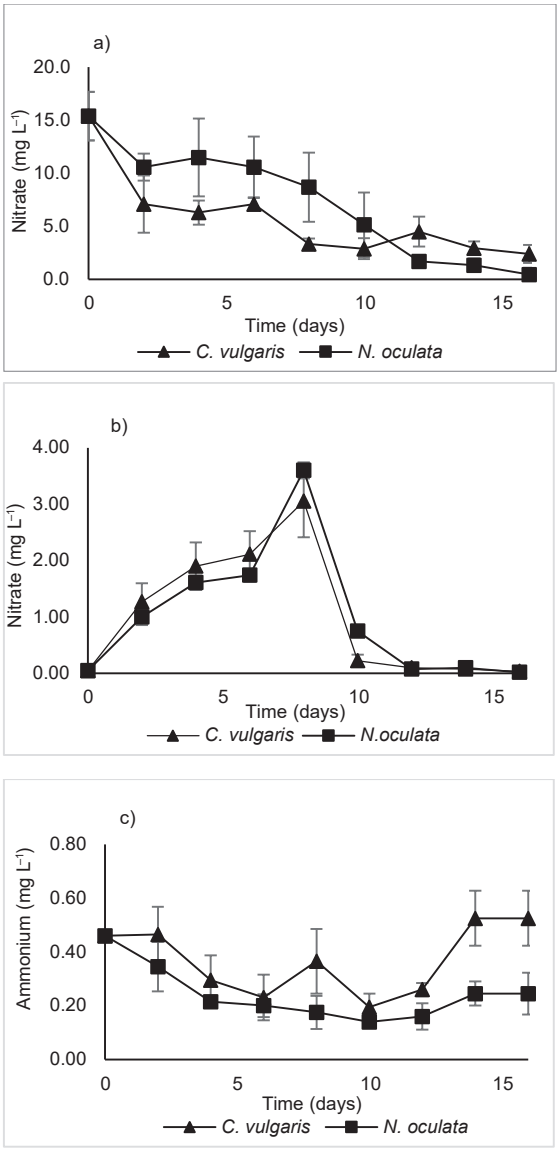

Fig. 5. Dynamic behavior of the concentration of a) nitrate, b) nitrite, c) ammonium with *C. vulgaris* and *N. oculata* grown in effluent at 72.9 mol m^{-2} s^{-1}.

does not work for all species and, in particular, it is not suitable for *C. vulgaris* but for *C. sorokiniana*.

Regarding COD, Chacón et al. (2004) could not establish a relationship between the removal of COD and the growth of microalgae, obtaining a removal of 54.8 % and associated it with the use of smaller culture volumes, in which the high cell densities reached also introduce the production of organic exoproducts, which do not allow differentiation between the organic matter existing in the residual water and that generated as a product of microalgae metabolism. Iriarte et al. (2007) mention that algae release exudates and particulate organic matter; Richmond (2004) adds that exudates inhibit algal growth significantly as a means to limit competition between species and as a defense against predation; it has been suggested that the production of autoinhibitory takes place in ultra-high-density algae mass cultures. The removal percentages achieved in this study are higher than those reported by Ferrer-Álvarez et al. (2015), except for phosphate, which, at the same time, coincides with a 0 % removal in the exponential phase in the case of nitrite. Paes et al. (2016), using a light intensity

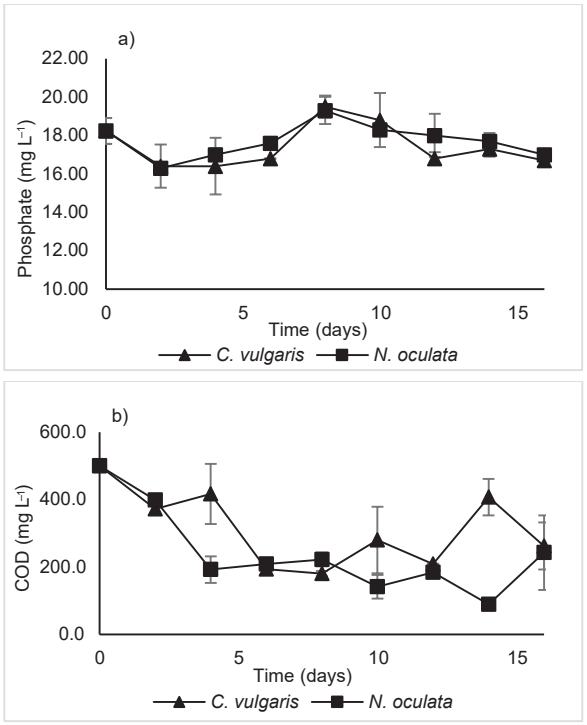

Fig. 6. Dynamic behavior of the concentration of a) phosphate y b) COD with *C. vulgaris* and *N. oculata* grown in effluent at 40.5 µmol m^{-2} s^{-1}.

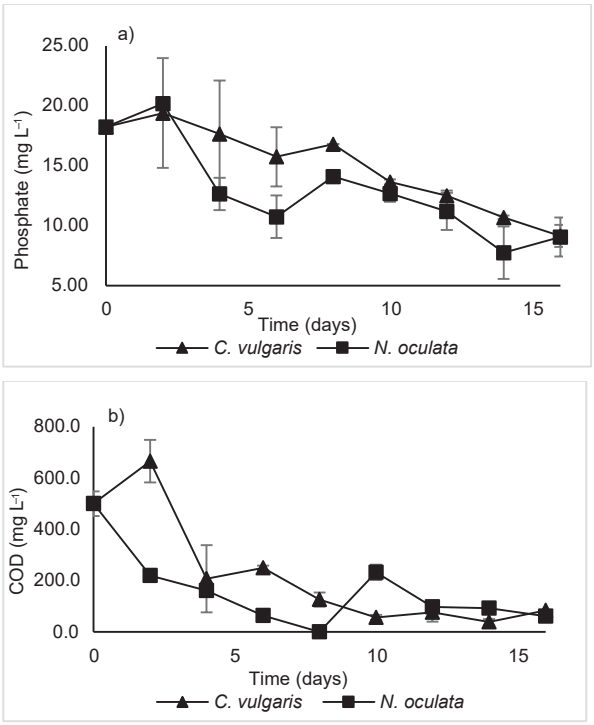

Fig. 7. Dynamic behavior of the concentration of a) phosphate y b) COD with *C. vulgaris* and *N. oculata* grown in effluent at 72.9 µmol m^{-2} s^{-1}.

of 350 µmol photons m^{-2} s^{-1}, report a phosphate consumption of 92.7 % in the established Conway medium, and approximately 34 % when there is nitrogen limitation, which supports the affirmation of Kube et al. (2018) that to increase the elimination of phosphorus it is necessary to increase the concentration of nitrogen. Haritz et al. (2017) report 89.5, 99.7, 92 and 75.5 % removals with nitrate, ammonium, phosphate and COD. Gil-Izquierdo et al. (2020) report a decrease of 89.90 and 99.70 % in nitrates and phosphates, respectively, using a consortium of microalgae (*Monoraphidium sp., Desmodesmus subspicatus, Nannochloris sp*.) in urban wastewater. With effluents from the laukaa fish, Calderini et al. (2021) report PO_4-P removals of 99 % with *M. griffithii* and Selenastrum sp, less than 75 % by *H. pluvialis*. In removing NO_3-N: less than 40% in the 3 species used (*Haematococcus pluvialis, Monoraphidium griffithii and Selenastrum sp*). Lopez-Sanchez et al. (2022b) carried out the treatment of water from the livestock sector (pigs, cattle, and poultry) with three microalgae *C. vulgaris, H. pluvialis, and Chlamydomonas reinhardtii*; the best result was with *C. vulgaris* as a monoculture in a digestate mixture of 0.125:0.4375:0.4375 (ADSW: ADPW: ADCW) reaching a total nitrogen removal of 85.00±1.58%, a total phosphorus removal of 65.69±3.05% and a chemical oxygen demand removal of 43.95 ±7.92%. Lopez-Sanchez et al. (2022a) mentions that *C. vulgaris* achieves removals of 25-99% of COD (chemical oxygen demand), 50-98% of total nitrogen, 41-95% of total phosphorus in swine wastewater; 45-82% COD, 89% total phosphorus in poultry wastewater; 62-92% COD, 81-94% total nitrogen, 85-94% total phosphorus in livestock wastewater and with *Nannochloropsis oculata*, he reports removals of 64-86 % of total nitrogen, 99 % of total phosphorus in wastewater from pig digests. Arguing that planting densities and the light used are not mentioned.

Production and productivity

Light intensity and growth phase significantly influenced biomass production (p=0.00000), but the combination of factors (Microalgae*Light intensity*Growth phase) showed no difference (p=0.62655). The highest production was obtained in the stationary phase at 72.9 µmol m^{-2} s^{-1} with 0.684 ±0.124 g L^{-1} and 0.718 ±0.122 g L^{-1} with *C. vulgaris* and

TABLE III. REMOVAL EFFICIENCY (%) OF NITROGENOUS AND PHOSPHATE COMPOUNDS AND ORGANIC MATTER IN TILAPIA EFFLUENTS (ETE2).

Microalgae	Light intensity (μmol m^{-2} s^{-1})	Growth phase	Nitrate (%)	Nitrate (%)	Ammonium (%)	Phosphate (%)	COD (%)
C. vulgaris	40.5	EXP	19.6 ±2.6	0	43.0 ±8.9	0	44.1 ±11.3
		STA	0	92.7 ±0.6	97.0 ±0.8	8.4 ±1.1	43.3 ±8.0
	72.9	EXP	79.7 ±11.7	0	57.2 ±10.2	25.2 ±1.5	89.0 ±3.8
		STA	83.2 ±9.8	22.5 ±3.5	0	49.9 ±5.2	82.9 ±5.1
N. oculata	40.5	EXP	19.9 ±3.0	79.6 ±9.3	17.9 ±0.1	0	71.7 ±6.9
		STA	0	79.2 ±11.3	92.1 ±1.1	8.0 ±3.2	51.4 ±12.7
	72.9	EXP	80.0 ±5.4	0	69.6 ±3.6	30.4 ±4.1	53.0 ±6.7
		STA	97.4 ±3.7	57.5 ±3.1	47.0 ±10.4	50.4 ±8.9	87.4 ±4.8

ETE2: *O. niloticus* rocky mountain var. Blanca sterile effluent; BBM: Bold's Basal Medium; EXP: Exponential; STA: Stationary.

N. oculata (**Table IV**), respectively. Therefore, the influence of light intensity and growth phase on biomass production and productivity was observed, being more significant than 72.9 μmol m^{-2} s^{-1} in the stationary phase with both microalgae. Production values are slightly higher than those obtained by Ferrer-Álvarez et al. (2015), who reached a yield of 0.598 mg L^{-1} in MBB and 0.319 mg L^{-1} in stationary phase effluent with *C. vulgaris*, with *N. oculata* they obtained 0.400 mg L^{-1} in MBB and 0.224 mg L^{-1} with effluent in stationary phase. Kumaran et al. (2023) report that *C. vulgaris* and 369 effluents from palm oil mills in the exponential phase (day 7) produced 0.42 g L^{-1}; however, with the control (BG-11 medium), they report a production of 0.34 g L^{-1} finding a higher production in the effluent with a light intensity of 400 μmol m^{-2} s^{-1}. Lopez-Sanchez et al. (2022a) make a compilation of the studies carried out in terms of production with different effluents, where it is mentioned that *C. vulgaris* reaches productions of 0.49-3.96 g L^{-1} in pig wastewater and greater than 1 g L^{-1} in poultry and cattle wastewater. With N. *oculata*, there are reports of 2.36-3.22 g L^{-1} in wastewater from pig digests. These yields are higher than those found in the present study. However, neither the light intensities used nor the seed cell density are mentioned, factors that can influence the growth of microalgae and, therefore, the amount of biomass produced. The productivity values found in this study with *C. vulgaris* are lower than those reported by Ferrer-Álvarez et al. (2015) in the exponential phase. They are within the values found in the stationary phase. The productivity values reported by Kumaran et al. (2023) are 0.060 g L^{-1} d^{-1} with effluent and 0.045 g L^{-1} d^{-1} with BG-11 medium, which is higher than what was found in this study where the light intensity used was lower. (72.9 μmol m^{-2} s^{-1}) to the one they used (400 μmol m^{-2} s^{-1}). It should be noted that the time the growth phases are reached influences productivity since the shorter the cultivation time, the more productivity is obtained.

Finally, the use of nutrients present in aquaculture effluents, based on the cultivation of microalgae, is environmentally responsible and sustainable, since it does not generate additional contaminants and provides an opportunity for the efficient recycling of nutrients. Furthermore, recent research has sought to increase the production and productivity of biomass, lipids, carbohydrates, pigments, fatty acid composition, photosynthetic performance,

TABLE IV. BIOMASS PRODUCTION AND PRODUCTIVITY IN *C. vulgaris* AND *N. oculata* UNDER TWO LIGHTING CONDITIONS IN TWO GROWTH PHASES IN ETE2.

Microalgae	Light intensity (μmol m^{-2} s^{-1})	Growth phase	Production (g L^{-1})	Productivity (g L^{-1} d^{-1})
C. vulgaris	40.5	EXP	0.152 ±0.044	0.015 ±0.004
		EST	0.297 ±0.017	0.019 ±0.001
	72.9	EXP	0.246 ±0.062	0.025 ±0.006
		EST	0.684 ±0.124	0.043 ±0.008
N. oculata	40.5	EXP	0.139 ±0.01	0.014 ±0.001
		EST	0.201 ±0.018	0.015 ±0.001
	72.9	EXP	0.423 ±0.08	0.042 ±0.008
		EST	0.718 ±0.122	0.048 ±0.008

ETE2: *O. niloticus* rocky mountain var. Blanca sterile effluent; BBM: Bold's Basal Medium; EXP: Exponential; STA: Stationary.

by manipulating light intensity between 60 and 2500 μmol m^{-2} s^{-1} (Khadim et al. 2018; Rebolledo-Oyarce et al. 2019; Pereira y Otero, 2019; Yuan et al. 2019; Farahin et al. 2021). Therefore, the use of nutrients from tilapia effluent in the cultivation of microalgae in optimal lighting conditions can be a renewable, sustainable, ecological, efficient and profitable alternative by reducing water consumption, redu-cing discharges and in production of biofuels from microalgae biomass (Markou and Nerantzis, 2013; Francavilla et al. 2015; Ferrer-Álvarez et al. 2015; Kichul et al. 2015; Pavón-Suriano et al. 2018; Xi et al. 2020; Maity and Mallick, 2022).

CONCLUSIONS

The results show that the time when the exponential and stationary phases are reached was not influenced by light, but cell growth was. The highest cell densities were at 72.9 μmol m^{-2} s^{-1} in the stationary phase. The combination of light intensity at 72.9 μmol m^{-2} s^{-1} and the stationary phase were better for removing nitrates, phosphates, and COD. Ammonium and nitrites obtained the highest removal efficiency at 40.5 μmol m^{-2} s^{-1}, also in the stationary phase. The greater removal of contaminants (nutrients) was influenced by light intensity, specifically nitrate, phosphate, and COD at 72.9 μmol m^{-2} s^{-1}. However, the microalga preferentially consumes ammonium and nitrite at low intensities of 40.5 μmol m^{-2} s^{-1}. Therefore, light intensity is an important factor to consider when there are high concentrations of pollutants. On the other hand, the light intensity of 72.9 μmol m^{-2} s^{-1} was also the most suitable for the highest production and productivity of biomass in the stationary phase.

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