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BIOMASS AND PIGMENTS PRODUCTION OF THE MIXED CULTURE OF MICROALGAE (*Hyaloraphidium contortum* AND *Chlorella vulgaris*) BY CULTIVATION IN MEDIA BASED ON COMMERCIAL FERTILIZER

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For the mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum*, was determined the effect of different sources of nutrients (Nitrofoska ®, Poliverdol ® and Guillard) on the production of photosynthetic pigments and biomass. Bioassays were performed in quadruplicate in batch cultures maintained in aeration and continuous illumination. The growth was determined by cell counting and the production of pigment by spectrophotometry. The cell density increased with time of planting, obtaining maximum production at 24 days with an average of 1.38 10^4± 5603.94; 1.09 10^4 ± 29.125 and 3.04 10^4± 8.900 cells mL^{-1} in Nitrofoska®, Poliverdol® and Guillard, respectively. The pigment (chlorophyll a and b and carotenoids) production was moderated (1.11 µg mL^{-1} at 24 days) in the medium Guillard during all-time research. Commercial agricultural fertilizers are an alternative source of nutrients for growth and pigment production in the mixed culture of microalgae.

Keywords: microalgae, fertilizers, growth, pigment biosynthesis

Introduction

Important parameters in regulating the growth of algae are: amount and quality of nutrients, light, pH, turbulence, salinity and temperature (Barsanti and Gualtieri, 2006). In biotechnological applications, microalgae have been investigated as a new source of food, energy products, agriculture, aquaculture and nutrition, emphasizing the use of its oil for biodiesel production (Chisti, 2007).

Microalgae become an alternative, highly efficient renewable green source, thanks to the ability to convert solar energy into useful chemical compounds, at a faster rate compared to other vegetable sources. One challenge consists in finding microalgae adapted to conditions of artificial cultivation and the biomass must have high lipid content and especially the omega-3 oils, omega-6, DHA (docosahexaenoic acid). On the other hand, the remainder, for example, the non lipid portion of microalgae, can be used to produce various industrial products based on carbohydrates such as ethanol or protein supplements, colorants, biofertilizer, antioxidants and wastewater treatments rich in nitrogen and phosphorus (Johnson, 2009).

Most commercial production of microalgae is aimed at healthy food market, mainly as tablets and capsules microalgae biomass (Raja *et al*., 2008). However, you should evaluate the microalgae cultures with the largest number of parameters related to determine the kinetics of growth and the potential for exploitation biochemical mass.

The aim of the present study was to investigate the effect of different sources of nutrients (Nitrofoska ®, Poliverdol ® and Guillard) on biomass and pigment production on mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum*.

Materials and methods

Microorganism and culture conditions

In this study a mixed culture of freshwater microalgae of *Chlorella vulgaris and Hyaloraphidium contortum* from microalgae collection of the Laboratory of Biology and cultivation of shellfish, Tropical Zoology Institute of the Central University of Venezuela was used. Strains were maintained in specific broth and solid agar slants media. The cultures were maintained in a chamber with controlled conditions, under a photoperiod of 12 hours light and 12 hours of darkness, with a light intensity of 45 µmol quanta $m^{-2}s^{-1}$ and an average temperature environment of 20 ± 2 ° C. The lighting was produced by lamps of 20 and 40 watts of day-light type. The microalgae were cultivated on three nutrient media, of which two were commercial inorganic fertilizers Nitrofoska® (Ammonium nitrogen (N) 6.5%, 5.5% Nitric Nitrogen, Phosphorus comparable (P) 12.0% Soluble Potash (K) 17.0% Magnesium 2.0% Sulphur 6.0%, Calcium 5.0%, 0.02% Boron and Zinc 0.01%) and Poliverdol ® (8-8-6 NPK with micronutrients for foliar spray. The media also contained small amounts of vitamin B1 and naphthylacetic acid. Other media are the traditional media Guillard with a final composition per liter of 75 mg KNO_3, 5.65 mg NaH_2PO_4 $2H_2O$, 4.360 mg EDTA Na_2, 3.150 mg $FeCl_3$ $6H_2O$, 0.010 mg $CuSO_4$ $5H_2O$, 0.022 mg $ZnSO_4$ $7H_2O$, 0.010 mg $CoCl_2$ $6H_2O$, 0.180 mg $MnCl_2$ $4H_2O$, 0.006 mg Na_2MoO_4 $2H_2O$, 2 mg crystalline cyanocobalamin (B12), 0.100 mg hydrochloric thiamine (B1) and 0.001 mg biotin crystalline (Stein, 1975). Quadruplicate cultures were initiated at a cell density of 5.0 10^4 cell mL^{-1} for each species of microalgae used in a container with a capacity of 1000 mL with 750 mL of sterile nutrient medium. Cultures were maintained at room temperature with

constant light source by two white fluorescent lamps of 40 W cool at an average distance of 20 cm, and aeration was supplied continuously by an electric pump of 110 W.

Biomass analysis and biochemical compound

Every 48 hours was evaluated algae growth for a period of 24 days by counting cells obtaining samples of about 5 mL of the culture fixed in an iodine solution. The counting was performed using a binocular microscope (Motic brand BA310Met, Hong Kong) and Neubauer hemacytometer (BC-Slide-5 Gadgetworkz, USA) at 0.1 mm deep, algal cells were counted in all of the boxes in the centre of the chamber, in both fields and then averaging the number of cells, and cell density was expressed as cell mL^{-1}. The kinetic parameters of algae growth, rate of multiplication (v) and doubling time of biomass (td) were also evaluated.

During the trial the pH, conductivity and total dissolved solids were measured using portable multiparameter equipment (Hanna brand HI1991301 model, Spain).

Soluble intracellular pigment extraction was performed using a solution of acetone: methanol (2:1 v/v) at 4 ° C overnight and was evaluated in each case directly on fresh cultures in triplicate. The pigment content was analyzed every 6 days and determined according to the formula proposed by Jeffrey and Humphrey (1975) for total chlorophyll; Strickland and Parsons (1972) for total carotenoids. Pigment concentration was expressed as mg mL^{-1} culture.

Statistical analysis

Data were analyzed using the Statgraphics plus v.5.1 software. After checking the assumptions of normality and homogeneity of variances, normality tests were applied to the analysis of variance data, in order to determine the effects of the independent variables: culture medium, time planting and their interactions on the dependent variable cell density (cells mL^{-1}), carotenoids, chlorophyll pigments (mg mL^{-1}) and physico-chemical parameters (conductivity, total dissolved solids and pH) using the SAS (Statistical Analysis Systems). Those variables with significant effects underwent Duncan test at 0.05 probability level.

Results and discussion

Physical and chemical parameters of the water

The electrical conductivity (S cm^{-1}), total dissolved solids (ppt) and the pH of the water using different culture media are shown in Figure 1. The highest values in electrical conductivity and total dissolved solids have been obtained initially for the medium based on Poliverdol®, while Guillard medium presented a higher pH. All these parameters tend to decrease over time. However for Nitrofoska® media, except the pH, were obtained minor values of all parameters compared to the other media used in this experiment.

Water conductivity depends on the presence, concentration and mobility of all ions present in it, and the charge or valence, relative ion concentrations and temperature

at which the measurement is made and it is important for their influence on water quality and biota.

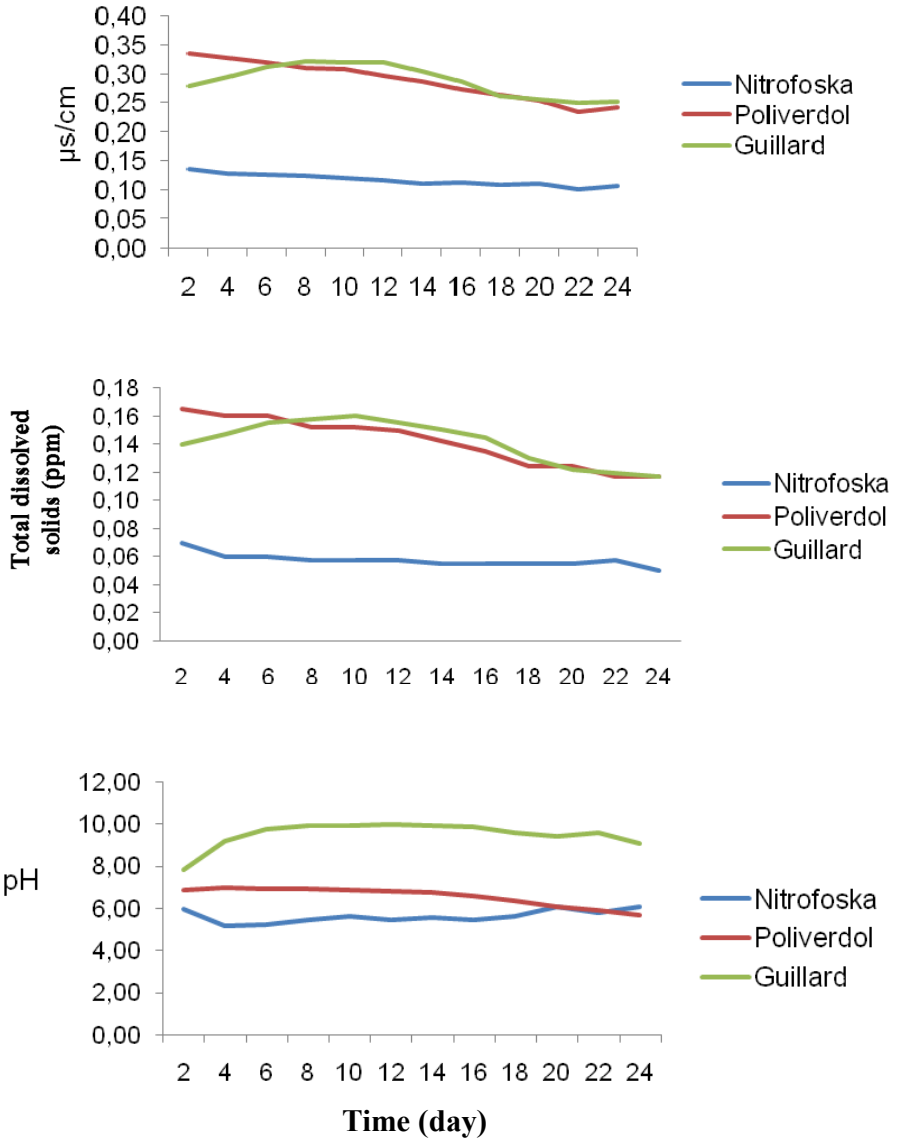

Figure 1. Electrical conductivity, total dissolved solids and pH dynamics during growth of mixed culture microalge *Hyaloraphidium contortium* and *Chlorella vulgaris* in media supplemented with commercial nutrients

In this experiment the electrical conductivity, total dissolved solids and pH remained in an acceptable range and their influence on the production of microalgae was reflected in the lower cell density found during cultivation on Nitrofoska® medium, probably because of the physical and chemical characteristics of the fertilizer. Silva-Benavides et al., (2008), recorded conductivities of rivers of Costa Rica on 35-300 microseconds cm^{-1}. Also, Perozzi *et al*. (2003) noted conductivities in the Vega Reservoir between 0.43 and 1.23 ms cm^{-1}. Furthermore, Jimenez and Ramos (1997) reported for the Carrillo Nabor Lake values of 2161 S cm^{-1}, being the allowed values of about 40 and 540 µs cm^{-1}. In 2006, the swamp of the Guájaro presented a high conductivity of 900 microsecond's cm^{-1}, with oxygen saturation and high dissolved solids (National University of Colombia, 2006). However, the values in this test are lower than those found by these authors as a result of using the preparation of culture media with distilled water, which reduces the ionic content, and sets a good water quality.

The high amount of dissolved solids was explained by the abundance of phytoplankton organisms (Roldan, 1992; Kacdle and Knight, 1996; Chapra 1997). However, the results in this study do not agree with the comments made by these researchers, because of the variation of the solids supply by culture media used and the use of ions by the microalgae.

The variation in the different nutrient sources (Guillard alkaline POLIVERDOL® neutral and slightly acidic Nitrofoska®) in culture media was one of the most important parameters influencing the microalgae growth and production of pigments. In Nitrifoska® medium the slightly acid pH slowed the cell growth due to the adaptation of the microorganisms to culture conditions, and afterwards the cell division was restored with the pH increase. Fuenmayor *et al*. (2009) observed pH variations without influencing the content of pigments in the marine cyanobacterium *Oscillatoria* MOF-06. Darly (1987) explained that the increase in microalgae biomass production increased the CO_2 consumption and decreasing the pH. Microalgae response to pH, varies widely with solubility of carbon dioxide and minerals in cultures, influencing directly or indirectly the metabolism of microalgae (Morris, *et. al*. 1999, Skoda, 1997).

Effect of interaction of planting time and source of nutrients on cell density in the mixed culture of Chlorella vulgaris and Hyaloraphidium contortum

The kinetics of mixed culture growth of *Chlorella vulgaris* and *Hyaloraphydium contortum* in the three sources of commercial nutrients used (Nitrofoska®, Guillard and Poliverdol®) showed a continuous increase in algal multiplication rate with culture age (Table 1). Different growth phases of variable duration depending on the medium used and environmental conditions were observed. Lacking cultivation lag period, the exponential growth phase ended after 6 days of incubation, cells divide regularly at a constant rate, achieving the cell concentration of 4.1 10^3, 6.3 10^3 and 6.4 10^3 cells mL^{-1} for culture media Nitrofoska®, Poliverdol® and Guillard, respectively. Subsequently, it was developed a growth phase delay, due to stationary cultivation conditions (reduced availability of nutrients in the culture

medium or environmental changes such as pH, temperature, light), resulting in reduced photosynthetic activity. In Nitrofoska® medium this phase was higher at 22 days with 1.34 10^4 cell mL^{-1}. For Poliverdol® it was obtained an average of 2.91 10^4 cell mL^{-1} and for Guillard 3.04 10^4 cell mL^{-1}, extending delay growth in the past two culture media to the end of the trial (24 days). The stationary phase in Nitrofoska® medium was reached between 22 and 24 days containing a cell density of 1.34 10^4cell mL^{-1} to 1.38 10^4 cell mL^{-1}, this occurred without population growth, due to the established balance between the growth rate and mortality rate. The typical decline phase of closed cultures was not reached in any of the culture media as a result of suitable environmental conditions, achieving sustained growth over time. The ANOVA detected a significant effect ($p < 0.05$) between the time of cultivation, medium composition and cell density.

Table 1. Effect of cultivation duration and medium composition on growth mixed culture of *Chlorella vugaris* and *Hyaloraphidium contortum* microalgae

Time of cultivation, day	**Average of cell density by cultivation on culture media supplemented with commercial nutriments, cells mL^{-1} 10^5**		
	Nitrofoska®	**Poliverdol®**	**Guillard**
0	0.001± 0.000	0.001± 0.000	0.001± 0.000
2	0.008 ± 0.001	0.01 ± 0.001	0.009 ± 0.002
4	0.02 ± 0.003	0.03 ± 0.006	0.025 ± 0.007
6	0.04 ± 0.014	0.06 ± 0.015	0.06 ± 0.014
8	0.05 ± 0.008	0.07 ± 0.029	0.06 ± 0.015
10	0.06 ± 0.020	0.10 ± 0.031	0.09 ± 0.023
12	0.07 ± 0.012	0.13 ± 0.042	0.12 ±0.044
14	0.08 ± 0.018	0.15 ± 0.065	0.13 ± 0.046
16	0.08 ±0.031	0.16 ± 0.035	0.14 ± 0.036
18	0.09 ± 0.033	0.22 ± 0.058	0.17 ± 0.049
20	0.13 ± 0.044	0.25 ± 0.056	0.24 ± 0.087
22	0.13 ± 0.047	0.28 ± 0.031	0.26 ± 0.080
24	0.14 ± 0.056	0.29 ± 0.109	3.04 ± 0.089

sd = Standard deviation

The algal density increased with the time of sowing and nutrient media, this was due to the accumulation of cells in time, exposed environmental conditions (light, temperature and nutrients) and genotypic and phenotypic characteristics of reproduction of the microalgae used. This is in agreement with the results reported by various authors who obtained an increase in cell density of *Chlorella* spp. *Synechococcus* spp. and *Synechocystis minuscule* with the inoculation time and production at 10, 12 and 24 days of 4.5 10^7 cells mL^{-1} 6.07 10^8 cells mL^{-1} and 1.50 10^8 cells mL^{-1}, respectively (Vera *et al*., 2004; Rosales *et al*., Jonte *et al.,* 2005, 2007). Also, Brito *et al*. (2006) found at 16 days values of 6.30 10^6 cells mL^{-1} in a mixed culture of *Selenastrum capricornutum* and *Chlorella vulgaris*. Moronta *et al.*

(2006) reported densities in *Chlorella sorokiniana* of 1.52 10^7 cells mL^{-1} and 1.6 10^7 cells mL^{-1} in autotrophic conditions after 15 days at pH 8.2 and 9.6. On the other hand, Romero (2011), obtained cell concentrations above 2 10^7 cells mL^{-1} for a retention time of six days, classified as a very good value for both controlled laboratory conditions and exterior conditions on crops of *Chlorella* spp. in wastewater from fish processing installations. Thus, Andrade *et al*. (2007) studied three fertilizer concentrations of triple 15 (1, 3 and 5 mL L^{-1} solution) and found the best conditions of growth for *Chlorella vulgaris*, in 1 mL concentration of Triple 15 medium after 20 days of cultivation. Moreover, Chacón *et al*. (2006) obtained for cultures of *Chlorella* spp. and *Scenedesmus* spp. in sterile distilled water and algal culture medium at a concentration of 6.0 mM and 0.3 mM of $NaNO_3$ and $NaPO_4$ respectively, productions of 1.80 10^7 and 4.69 10^6 cells mL^{-1}, in both media. Likewise, Rosales-Lowe *et al*. (2008) obtained the highest cell density to a renewal fee of 5% to 1.55 and 1.49 10^7 cells mL^{-1} for high and low light using marine microalgae *Dunaliella viridis* cultivated in algal medium with a concentration of 6.0 mM of $NaNO_3$. Finally, Prieto *et al*. (2005) found significant differences for the effect of the media (Guillard, Rither and Conway) on population growth of diatoms *Actynociclus normanii, Cyclotella glomerata* and *Neodelphyneis pelagica*, whose concentrations reached 2.67 10^2± 277.77 cells mL^{-1}, 1.6 ± 69.68 cells mL^{-1} and 2.7 ± 49.180 cells mL^{-1}, respectively, and higher biomass production was obtained in Guillard medium.

Kinetics growth parameters of the mixed culture of Hyaloraphidium contortum-Chlorella vulgaris

The growth rates in the exponential phase of culture were 1.34, 1.01 and 1.30 cell day^{-1}, with cell generation times of 0.74, 0.99 and 0.77 days by stationary cultivation in the media Guillard Poliverdol® and Nitrofoska®, respectively (Table 2). It was obtained lower cell density (4.1 10^3 cell mL^{-1}) in the inorganic fertilizer foliar Nitrofoska® at a higher growth rate and shorter doubling time compared to the Poliverdol® media. This results is possibly due to differences in physical and chemical characteristics of the Nitrofoska® fertilizer, which presents a slightly acidic pH, lower electrical conductivity and total dissolved solids (Figure 1) as well as low nutrient availability, causing a decrease in the ability of reproduction of microalgae and altering the growth kinetics. Microalgae growth is limited by several factors such as the type and concentration of nutrients, pH, light intensity, photoperiod, genetic deficiency and CO_2 (Roth and Buerguer, 1987 Molina *et al*. 1995; Fábregas *et al.* 1996) all this modifies the biochemical composition of microalgae (Goldman, 1980). Brito *et al*. (2006) reported exponential growth phase after 4 days of seeding with a mixed culture of *Chlorella vulgaris* and *Selenastrum capricornutum*. These authors reported that the average cell density was 3.6 10^6 and 7.8 10^5 cell mL^{-1}, with a growth rate of 0.95 and 1.37 cell day^{-1} and a generation time of 1.05 and 0.72 days by cultivation in nutrient sources Nitrofoska® and Guillard, respectively. The results mentioned above are similar to our results in terms of growth rates and doubling time.

Table 2. Estimation of growth kinetic parameters on mixed culture of *Chlorella vulgaris-Hyaloraphidium contortium* by stationary cultivation in supplemented media

Commercial product for culture medium supplementation	Time of cultivation, days	Cell density x10^3, cell mL^{-1}	v, cell day^{-1}	td, day
NITROFOSKA®	0	0.1		
	2	0.87	3.21	0.32
	4	1.68	0.95	1.05
	6	4.14	1.30	0.77
POLIVERDOL®	0	0.1		
	2	1.17	3.54	0.28
	4	3.15	1.43	0.70
	6	6.35	1.01	0.99
GUILLARD	0	0.1		
	2	0.92	3.20	0.31
	4	2.52	1.45	0.69
	6	6.40	1.34	0.74

v = growth rate, day^{-1}; td = biomass doubling time, day

Photosynthetic pigments production

Effect of the duration of cultivation on pigments biosynthesis

In this research the photosynthetic pigments were independently influenced by time of cultivation and culture medium composition. In Nitrofoska® medium, the highest concentrations of intracellular pigments were obtained at 24 days with average values of 0.65, 0.35 and 0.38 µg mL^{-1} of chlorophyll a, b and total carotenoids, respectively. In the culture media Poliverdol® and Guillard, higher intracellular pigment content was found at 18 days of cultivation with values of 2.21 and 3.06 µg mL^{-1} of chlorophyll a 0.73 and 1.10 µg mL^{-1} of chlorophyll b and 0.85 and 1.04 µg $mL^{-1}$of total carotenoids (Table 3). The analysis of variance detected a significant influence ($p < 0.05$) of the time in the contents of chlorophyll a in the media Poiverdol® and Guillard. In Nitrofoska® media did not detect significant differences between the time ($p < 0.1530$) and chlorophyll a. Regarding the concentration of chlorophyll b, we found a significant effect between time ($p < 0.05$) and the means Nitrofoska®, Poliverdol® and Guillard. The analysis of variance showed a significant effect ($p < 0.05$) of time in the production of total carotenoids.

Effect of growth medium composition on the yield of the pigments

The photosynthetic pigments are highly valued in the food industry as they are responsible for the color of fruits and vegetables and because of their importance to the physiological and dietary (Melendez *et al*., 2004).

Table 3. Yield of pigments production in mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum* microalgae in correlation with duration of cultivation using commercial nutrients

Comercial product for culture medium supplementation	Time of cultivation, day	Pigment production , µg mL^{-1}		
		Chlorophyll a	Chlorophyll b	Total carotenoids
Nitrofoska®	6	0.41 ± 0.13 a	0.23 ± 0.10 b	0.19 ± 0.02 c
	12	0.55 ± 0.15 a	0.35 ± 0.07 a	0.32 ± 0.06 b
	18	0.63 ± 0.15 a	0.16 ± 0.05 b	0.26 ± 0.06 c
	24	**0.66** ± 0.15 a	**0.35** ± 0.07 a	**0.38** ± 0.12 a
Poliverdol®	6	0.62 ± 0.10 c	0.29 ± 0.06 b	0.29 ± 0.04 c
	12	1.60 ± 0.72ba	0.68 ± 0.26 a	0.60 ± 0.25 b
	18	**2.21** ± 0.55a	**0.73** ± 0.23 a	**0.85** ± 0.23 a
	24	0.90 ± 0.18bc	0.36 ± 0.02 b	0.53 ± 0.09 cb
Guillard	6	0.98 ± 0.18b	0.42 ± 0.09b	0.39 ± 0.02b
	12	2.13 ± 0.67ba	0.92 ± 0.32ba	0.79 ± 0.06a
	18	**3.07** ± 1.42a	**1.10** ± 0.50a	1.04 ± 0.34a
	24	2.65 ± 1.48ba	1.07 ± 0.48a	**1.11** ± 0.12a

Number of observations = 4; Means with the same letter are not significantly different from each other; letters a, b, c indicate differences compared to the control by considering the Duncan test, $p < 0.05$.

Regarding the source of nutrients, the Guillard medium provides the nutrients for mixed culture, qualitatively and quantitatively, obtaining a higher growth rate and a higher concentration of pigments compared to agricultural fertilizers. However, Poliverdol® medium showed a higher content of pigment production than Nitrofoska ® (Table 4). Overall microalgae modified biochemical responses due to factors such as quantity and quality of nutrients, light, temperature and pH, which may have influenced the production of pigments in different growth media.

The production of pigments in microalgae varies widely between organisms and culture media used, achieving yields between 2.19 and 9.52 µg mL^{-1} of chlorophyll in *Chlorella* spp. and *Scenesdemus* spp. respectively (Loreto *et al*. 2003; Chacon *et al*. 2006). Guevara *et al*. (2005) reported a production of carotenoids of 38.40 ± 1.92, 32.8 ± 1.97 and 21.00 ± 0.84 µg mL^{-1} for cultures of *Dunaliella* spp. on commercial algal media at 30 days of age. Instead, Chacón *et al*. (2006) obtained 4.95 ± 0.54 µg mL^{-1} of total carotenoids in stationary phase culture with *Chlorella* spp. These values are higher than those obtained in this study. Loreto *et al*. (2003) determined values of 12.6 ± 3.2 to 15.7 ± 1.2 µg mL^{-1} of chlorophyll a and total carotenoids from 1.2 ± 0.3 to 4.5 ± 0.3 µg mL^{-1} in

Anabaena PCC 7120, controlling the medium nitrogen composition. Furthermore, Andrade *et al.* (2009) obtained 1.11 ± 0.03 µg mL^{-1} of totals carotenoids of *Scenedesmus* spp. cultivation in stationary phase (15 days) and using Nitrofoska® as culture medium. The yields of total carotenoids in this study were similar to those reported by Jonte *et al*. (2007) and Molina *et al*. (2007) in the cianobacteria *Synechocystis minuscule* and *Rhodosorus marinus*, with values ranging from 1.66 to 2.17 µg mL^{-1} and 0.34 ± 0.18, 3.66 ± 0.54 and 0.17 ± 0.02 µg mL^{-1} after 15 days of cultivation, respectively. Generally, the production of photosynthetic pigments reported by different authors differ due to the experimental biotechnological conditions, the phytoplankton species and the cultivation environmental conditions.

Table 4. Chlorophyll a, b and total carotenoids (µg mL^{-1}) production in mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum* on three nutrient sources, after 24 days of stationary cultivation

Comercial product for culture medium supplementation	Chlorophyll a	Chlorophyll b	Total carotenoids
Nitrofoska®	0.56 ± 0.13c	0.28 ± 0.11c	0.28 ± 0.10c
Poliverdol®	1.34 ± 0.10b	0.51 ± 0.25b	0.57 ± 0.26b
Guillard	2.20 ± 0.18a	0.88 ± 0.44a	0.83 ± 0.40a

Number of observations=16; Means with the same letter are not significantly different from each other; letters a, b, c indicate differences compared with the control by considering the Duncan test, $p < 0.05$.

Conclusions

Microalgae growth was influenced by the biotechnological conditions of cultivation, especially by the time of cultivation, the type and quantity of the nutrient sources, presenting a higher cell density with Guillard and Poliverdol® inorganic fertilizer. However, the production of photosynthetic pigments responds independently to the effect of time and nutrient source. In case of the mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortium* the foliar inorganic fertilizer Poliverdol® was considered a suitable source of nutrients compared to Nitrofoska®, representing an alternative for culture medium supplementation for algae cultivation in order to obtain biomass, freshwater phytoplankton or pigments, with great impact from the economical point of view and feasibility.

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References

Andrade, E. R., Torres, R., Montes, E.J., and Fernández, A.C. 2007. Obtención de harina apartir del cultivo de *Chlorella vulgaris* y su análisis proteico. *Temas Agrarios*, **12**(1), 5057.

Andrade, E. R., Vera, A. L., Cárdenas, C. H., and Morales, E. D. 2009. Producción de biomasa de la microalga *Scenedesmus* spp. utilizando aguas residuales de pescadería. *Revista Técnica de la Facultad de Ingeniería Universidad del Zulia*, **32**(2), 126-134.

Barsanti, L. and Gualtieri, P. 2006. Algae. *Anatomy, Biochemistry, and Biotechnology***.** *Taylor & Francis group*. 159-301.

Brito, D., Milani, N., Pereira, G., González, M., and Moran, R. 2006. Crecimiento de microalgas de agua dulce, en dos medios de cultivo Guillard y un fertilizante comercial Nitrofoska. *Revista Cientifica de la Facultad Experimental de Ciencias de la Universidad del Zulia*. **14** (4), 394-410.

Chacón, C., Andrade, C., Cardenas, C., Araujo, I., and Morales, E. 2006. Uso de *Chlorella* sp. y *Scenedesmus* sp. en la remoción de Nitrógeno, Fósforo y DQO de Aguas Residuales Urbanas de Maracaibo, Venezuela. *Boletín del Centro de Investigaciones Biológicas*, **38**(2), 94-108.

Chapra, S.1997. *Surface water-quality modeling*. Mc Graw-Hill. USA.

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances*, **25**, 294-306.

Darly, W. M. 1987. *Biología de las algas. Enfoque fisiológico*. Limusa S.A., México.

Fábregas, J., Patiño, M., Morales, E., Cordero, B., and Otero, A. 1996. Optimal renewal rate and nutrient concentration for the production of the marine microalgae *Phaeodactylum tricornutum* in semi-continuous cultures. *Applied and Enviromental Microbiology*, **62**, 266-268.

Fuenmayor, G., Jonte, L., Rosales-Loaiza, N., and Morales, E. 2009. Crecimiento de la cianobacteria marina *Oscillatoria* sp. MOF-06 en relación al pH en cultivos discontinuos. *Revista de la Sociedad Venezolana de Microbiología* (RSVM), **29**, 21-25.

Guevara, M., Lodeiros, C., and Gómez, O. 2005. Carotenogénesis de cinco cepas del alga *Dunaliella* sp. (Chlorophyceae) aisladas de lagunas hipersalinas de Venezuela. *Revista Biología Tropical*, **53**(3-4), 331-337.

Goldman, J. 1980. Physiological aspects in algal mass culture, In: *Algae Biomass: Production and use*. Shelefand G., Soeder C., (eds) 343-360 p.

Jeffrey, S., and Humphrey, G. 1975. New spectrophotometric equations for determining chlorophylls a, b, c, and c_2 in higher plants, algae and natural populations. *Biochemical Physiology Pflanzen*, **167**, 191-194.

Jiménez, C. B., and Ramos H. J. 1997. Revisión de los criterios ecológicos de calidad del agua para uso agrícola. Elaborado para la Comisión Nacional del Agua. Instituto de Ingeniería, UNAM.

Johnson, M. 2009. Microalgal Biodiesel Production through a Novel Attached Culture System and Conversion Parameters. Tesis de Maestría. Universidad de Virginia Blacksburg, USA.

Jonte, L., Rosales, N., Yepez, M., Briceño, B. and Moralez, E. 2007. Respuesta de la cianobacteria *Synechocystis minúscula* a sustratos orgánicos en condiciones mixotróficas. *Boletín del centro de investigaciones biológicas*, **41** (1), 1- 14.

Kacdle, R., and Knight, R. (1996). *Treatment wetlands*. Ed. CRC Press. USA.

Loreto, C., Rosales, N., Bermúdez, J., and Morales, E. (2003). Producción de Producción de pigmentos y proteínas de la cianobacterias *ANABAENA* PCC 7120 en relación a la concentración de nitrógeno e irradiancia. *Revista Guayana Botánica*, **60** (2), 83 – 89.

Meléndez, A., Vicario, I., and Heredia, F. 2004. Importancia nutricional de los pigmentos carotenoides. *Archivos Latinoamericanos de Nutrición*, **54** (2), 149- 154.

Molina, E., Sánchez, J., García, F., Fernández, J., Acién, F., and Urda J. 1995. Biomass and icosapentaenoic acid productivities from an outdoor batch culture of *Phaeodactylum tricornutum* UTEX 640 in an airlift tubular photobioreactor. *Applied Microbiology and Biotechnology*, **42**, 658-663.

Molina, L., Jonte, R., Mora, J., Ortega, J. and Morales, E. 2007. Influencia de la salinidad sobre el crecimiento de la microalga *Rhodosorus marinus* (Rhodophyta) en cultivos discontinuos. *Revista Facultad de Agronomía (LUZ)*, **24** (1), 249- 253.

Moronta, R., Mora, R., and Morales, E. 2006. Respuesta de la microalga *Chlorella sorokiniana* al pH, salinidad y temperatura en condiciones axénicas y no axénicas. *Revista Facultad de Agronomía (LUZ)*, **23**, 27-41.

Morris, H., Quintana, M., Almarales., A., and Hernández., L. 1999. Composición bioquímica y evaluación de la calidad proteica de la biomasa autotrófica de *Chlorella vulgaris*. *Revista Cubana de Alimentación y Nutrición,* **13**(2), 123-128.

Perozzi, F. L., Ortiz, M. M., and López, L. E. 2003. Efecto del agua del embalse de la Vega en la lipoperoxidación y los niveles de la acetilcolinesterasa en el hígado y el músculo de *Xiphophorus helleri*. *Revista Internacional Contaminación Ambiental*, **19**(3), 145-155.

Prieto, M., Mogollón, M., Castro, A., and Sierra, L. 2005. Efecto del medio y condiciones de cultivo en la productividad de tres diatomeas marinas con potencial acuícola. *Revista MVZ Córdoba*, **10** (1), 554 – 568-

Raja, R., Hemaiswarya, S., Ashok, N., Sridhar, S. and Rengasamy, R. 2008. A Perspective on the Biotechnological Potential of Microalgae. *Critical Review Microbiology*, **34**,77–88.

Roldán, G. 1992. *Manual de limnología Neotropical*. Universidad de Antioquia, Medellín.

Roth, P., and Buerguer, S. 1987. Mixotrophic cultivation of *Chlorella vulgaris* homocontinuos cultivation in chemostat. *Acta Biothecnology*, **7**,17-21.

Romero, L. T. 2011. Desarrollo de *Chlorella* spp. en riles orgánicos pesqueros y su influencia en la remoción de la contaminación. *Ingeniería Hidráulica y Ambiental*, **XXXII** (3), 32-38.

Rosales, N., Ortega, J., Mora, R. and Morales, E. 2005. Influencia de la salinidad sobre el crecimiento y composición bioquímica de la cianobacteria *Synechococcus* sp. *Revista de Ciencias Marinas,* **31** (2), 349- 355.

Rosales-Loaiza, N., Avendaño, D., Otero, A and Morales, E. 2008. Crecimiento, producción de pigmentos y proteínas de la microalga *Dunaliella viridis* (Chlorophyta) en cultivos semicontinuos. *Boletín Del Centro De Investigaciones Biológicas*, **42**(3), 323–444.

Silva-Benavides, A. M., Sili, C., and Torzillo, G. 2008. Cyanoprocaryota y microalgas (Chlorophyceae y Bacillariophyceae) bentónicas dominantes en ríos de Costa Rica. *Revista Biología Tropical*, **56** (Suppl. 4), 221-235.

Skoda, B. 1997. Contributions to the biochemical taxonomy of the genus *Chlorella b*eijerincks. l. pigment composition. *Algological Studies*, **87**, 109-136.

Stein, J. 1975. Handbook of phycological methods. Culture methods and growth measurements. Cambriedge University Press.

Strickland, J. and Parsons, T. 1972. A practical handbook of seawater analysis. *Fisherie Research Board of Canadá*. Bulletin **167** Ottawa. Pp. 310-318.

Universidad Nacional de Colombia 2006. Estudios e investigaciones de las obras de restauración ambiental y de navegación del Canal del Dique. Bogotá.

Vera, A., Martínez, M., Morillo, K. and Montes, S. 2004. Cultivo discontinuo de *Chlorella* spp. en medios enriquecidos con el exudado gomoso de *Acacia macracantha.* Boletín del Centro de Investigaciones Biológicas, **38** (2), 1 – 10.