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Use of alternative media and different types of recipients in a laboratory culture of *Ankistrodesmus gracilis* (Reinsch) Korshikov (Chlorophyceae)

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ABSTRACT. A laboratory culture of *Ankistrodesmus gracilis* algae was evaluated by studying the biology of the species and its chemical composition in a traditional medium (CHU_{12}) and in two alternative culture media, NPK (20-5-20) and macrophyte (*Eichhornia crassipes*) + NPK, in three different types of recipients (fiberglass, carboy and plastic bag). First peak in the growth curve of *Ankistrodesmus gracilis* occurred on the ninth day in macrophyte + NPK medium (74.16 x 10^5 cells mL^{-1}) in a fiberglass recipient. However, highest density ($p < 0.01$) was reported in medium CHU_{12} (122.87 x 10^5 cells mL^{-1}) in a plastic bag on the twelfth day. Cell density was over 70 x 10^5 cells mL^{-1} starting on the twelfth day. Growth rate of *A. gracilis* was similar ($p > 0.05$) in culture media in the three recipients. Protein and fiber were similar ($p > 0.05$) in the treatments, but lipids were higher ($p < 0.05$) in NPK. Nitrate, ammonia, total phosphorus and orthophosphate contents were over 1 mg L^{-1} in NPK ($p < 0.01$). Results show that alternative media, such as NPK and macrophyte + NPK, are possible for large-scale culture of *A. gracilis* cultured in three types of recipients. Costs are low, occupying less space when cultured in plastic bags and in the laboratory.

Keywords: algae, macrophyte medium, NPK, CHU_{12}, biochemical composition, growth.

RESUMO. Utilização de meios alternativos e diferentes tipos de recipientes no cultivo de *Ankistrodesmus gracilis* (Reinsch) Korshikov (Chlorophyceae) em laboratório. O objetivo do estudo foi avaliar os aspectos biológicos e a composição química da alga *Ankistrodesmus gracilis* em laboratório utilizando um meio tradicional (CHU_{12}) e dois meios alternativos, NPK (20-5-20) e macrófita (*Eichhornia crassipes*) + NPK em três diferentes tipos de recipientes (cuba de fibra de vidro translúcido, garrafões e saco plástico). O primeiro pico de densidade celular de *Ankistrodesmus gracilis* ocorreu no nono dia da curva de crescimento em meio macrófita+NPK (74,16 x 10^5 células mL^{-1}) no recipiente de fibra de vidro, porém a maior densidade ($p < 0,01$) foi observada no meio CHU_{12} (122,87 x 10^5 células mL^{-1}) em saco plástico no décimo segundo dia, a partir do qual a densidade celular permaneceu acima de 70 x 10^5 células mL^{-1}. A taxa de crescimento de *A. gracilis* foi similar ($p > 0,05$) nos três recipientes e meios de cultivo. Os teores de proteína e fibra foram similares ($p > 0,05$) nos tratamentos utilizados, já os de lipídios foram mais elevados ($p < 0,05$) no meio NPK. Os teores médios de nitrato, amônia, fósforo total e ortofosfato estiveram acima de 1 mg L^{-1} no meio NPK ($p < 0,01$). Os resultados obtidos neste estudo indicam a possibilidade do uso de meios alternativos como o NPK e macrophyte +NPK para o cultivo de *A. gracilis* em larga escala cultivados nos três tipos de recipientes, porém, em saco plástico o custo é baixo e ocupa menos espaço em cultivo de laboratório.

Palavras-chave: alga, meio de macrófita, NPK, CHU_{12}, composição bioquímica, crescimento.

Introduction

Grown microalgae are widely used in aquaculture as a preferred natural feed for zooplankton and fish larvae. Despite the efforts made to replace microalgae with inert diets, aquaculture still depends on the production and use of live food for important aquatic animals. The fresh water microalgae *Ankistrodesmus gracilis* (Reinsch) Korshikov is one of the live foods most frequently employed in the aquaculture system.

Numerous algae species belonging to the Chlorophyceae family have been reported to contain high levels (25-50% dry weight) of neutral lipid. This fact suggests that this class of algae may

represent a large pool of organisms from which initial feed for zooplankton and fish larvae could be obtained (SOUTO et al., 2008).

Laboratory culture of fresh water microalgae as feed for water organisms has been on the increase, even though certain problems still remain unsolved. These comprise production costs, nutritional value of live feed, adequate space-saving recipients for culture, appropriate management and production in the shortest time possible.

The carboys or small bag system and fiberglass containers are recipients employed in culture systems that directly affect growth and production costs. Maximizing production while minimizing costs is of the greatest interest to all involved in algae culture.

In the case of production costs, the alternative culture medium, such as NPK (20-5-20), has been employed with great production of *A. gracilis*, featuring high nutrition rates (SIPAÚBA-TAVARES; ROCHA, 1993; HARDY; CASTRO, 2000; SIPAÚBA-TAVARES; PEREIRA, 2008).

The use of inorganic fertilizers is simple, since they are widely available, dissolve easily, feature a defined composition, high nitrogen and phosphorus rate, and trigger moderate pH in the medium (TEW et al., 2006). *Ankistrodesmus gracilis* grown in medium NPK (20-5-20) has high photosynthetic rates, with a protein content of approximately 47% dry weight (SIPAÚBA-TAVARES; PEREIRA, 2008).

Vitamins have also been added to alga culture and result in a significant increase. Complex B vitamins (thiamine, cobalamin and biotin) are essential for most microalgae, since vitamin B_{12} is required by approximately 70% of plankton algae (HOFF; SNELL, 1997).

Better alga yield with low production costs in large-scale culture is one of the aims of aquaculture worldwide. This is due to the fact that microalgae produce fatty acids, generally contain high levels of water soluble vitamins, source of carbohydrate and proteins to be used as food and feed for fish larvae and zooplankton (LIN et al., 2007; SOUTO et al., 2008).

Three culture media (NPK, macrophyte + NPK, and CHU_{12}) in different types of recipients (fiberglass, carboy, and plastic bag) were employed in the current study for the lab culture of *A. gracilis*. The current experiment evaluates the effect of culture medium and recipients in the growth of *A. gracilis*, with special reference to its biology, nutritional value and effect of culture water quality in the lab development of the microalgae.

Material and methods

Algae culture

The strain of green algae *Ankistrodesmus gracilis* was obtained from the Algae Physiology Laboratory (005CH), Universidade Federal de São Carlos-UFSCar, originally isolated from Broa Reservoir, São Paulo State, Brazil. Algae were batch-cultured in the laboratory at 22 ± 2ºC, in a light regimen between 51.2 and 78.1 μE cm^{-2} s^{-1} provided by white fluorescent daylight tubes (Philips), on a 24h light cycle, and bubbled with air. Start culture was grown in 2 L flasks containing NPK medium. The current study comprises three culture media, CHU_{12}, NPK (SIPAÚBA-TAVARES, 1995), and a third medium with a mixture of macrophytes and NPK medium. The latter contained approximately 5 kg of *Eichhornia crassipes*, which were ground and boiled in distilled water for 1h. The hot extract was filtered and autoclaved at 120ºC, during 20 min. A 70 mL sub-sample was retrieved after cooling and completed up to 1,300 mL of distilled water. Further, 2.5 mL NPK and 100 mL inoculum of *A. gracilis* were added to obtain an approximate density of 3 x 10^5 cells mL^{-1}. When cultures reached the late exponential growth phase (day-8), their content was transferred to sterilized recipients containing 13 L with a density of 4.4 x 10^5 cells mL^{-1} for medium NPK; 5.1 x 10^5 cells mL^{-1} for macrophyte + NPK medium; and 4.5 x 10^5 cells mL^{-1} for medium CHU_{12}, enriched filtered fresh water (1 and 5 μm). Complex B vitamins were added to the media, at the rate of 0.01 g L^{-1}, following Sipaúba-Tavares (1995). Three different types of recipients were used for the cultivation of *A. gracilis* in the laboratory, namely, glass fiber, carboy and plastic bag (Figure 1). The experiment was undertaken in triplicate during 22 days. Mean cell size was 18.42 x 3.3 μm.

Growth

Duplicate 1 mL aliquots were removed daily from the algae culture and a minimum of 2 x 1 μL sub-sample were used for cell quantification by a Neubauer hemocytometer. Growth rate (k) was obtained using the exponential growth phase, which represented the number of cell divisions per day. Doubling time, also called division time or generation time, was calculated from results obtained from the growth rate, and may be calculated as follows:

$Td = 1\ k^{-1}$ or $Td = 24\ k^{-1}$

where:

Td = duplication time; $1\ k^{-1}$ = days per division, and $24\ k^{-1}$ = hours per division.

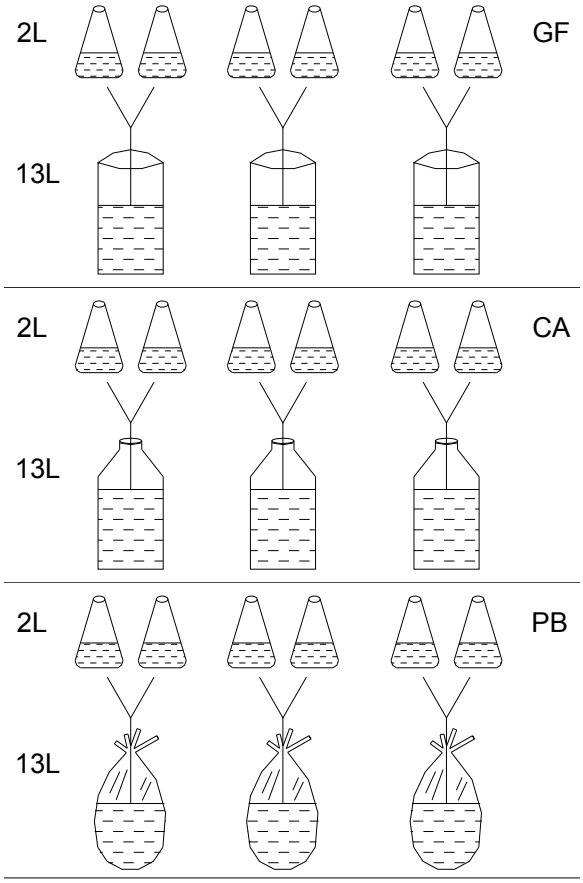

Figure 1. Schematic diagram of 13-L recipients for *Ankistrodesmus gracilis* culture, where: GF= glass fiber; CA= carboy and PB= plastic bag.

Algae characteristics

Dry weight was determined by obtaining 10 mL of each replication, collected twice a week and filtered in fiberglass filter (GFC 0.7 μm pore size), previously washed in distilled water. Further, the filter was dried at 60ºC and weighted till constant weight. The material was incinerated in an oven at 500°C, for 4 hours, to calculate algae ash contents.

Total length (μm) of 50 specimens from each culture medium was determined using an Olympus BX 50 microscope, with images analysis system Pro Plus 4.1, Media Cybernetics, USA, with a 400x micrometric objective. Calculation of cell volume was undertaken by mean cell size with the use of the most appropriate geometric form, which corresponds to the formula of two coupled cones (VOLLENWEIDER, 1974; BOTTREL et al., 1976).

Total organic carbon per cell was calculated from the relationship between carbon content and cell volume for fresh water algae. It can be calculated as follows:

$$C=0.1204 \times V^{1.051}$$

where:

C= organic content in pg cell^{-1}; V= cell volume.

Biochemical composition

At the end of the experiment with *A. gracilis*, culture media were concentrated in a skimmer and lyophilized for the analysis of proteins; lipids and fiber were determined following AOAC (1990).

Hydrological data

Several hydrological variables were analyzed to evaluate the effect of water quality on *A. gracilis* culture. Water samples were monitored twice a week in all recipients, with a glass bottle (500 mL). Dissolved oxygen and inorganic carbon were analyzed according to Golterman et al. (1978). The pH, temperature and conductivity were measured with a Corning PS-17 pH meter, Corning PS-16 temperature meter and Corning PS-15 conductivity meter. Total phosphorus, orthophosphate, ammonia, nitrite and nitrate were determined according to techniques described by Golterman et al. (1978) and Koroleff (1976). Chlorophyll-*a* was evaluated following Nusch (1980).

Statistical analyses

The life history and hydrological characteristics of the water culture of algae *Ankistrodesmus gracilis* were analyzed by two-way ANOVA for simple verification, taking into account recipients and culture media (FOWLER et al., 1998). Significance level for statistical test results was $p = 0.05$.

Results

Exponential increase in the growth curve of *Ankistrodesmus gracilis* occurred on the 9th day in the macrophyte + NPK medium (74.16 x 10^5 cells mL^{-1}) in the glass fiber recipient. However, the highest number of cells was reported in medium CHU_{12} in a plastic bag (122.87 x 10^5 cells mL^{-1}) on the 12th day. *A. gracilis* cell density remained above 70 x 10^5 cells mL^{-1} in medium CHU_{12} from the 12th day. From the 19th day, algae tended to decrease and reached a minimum of 4.74 x 10^5 cells mL^{-1} on the 22nd day in NPK medium in the plastic bag. Cell density was significantly higher ($p < 0.01$) in CHU_{12} (Figure 2, Table 1).

The growth rate varied between $k = 0.14$ and 0.46 in the three media and recipients used ($p > 0.05$), with the highest k rate in NPK ($k = 0.46$) in a plastic bag recipient (Table 1).

Cell doubling time ($p > 0.05$) varied between 2.16 and 7.08 days in the treatments employed. Cell

volume was higher in NPK medium ($p < 0.01$) although similar rates were given in macrophyte + NPK and CHU_{12} media. Mean size did not vary ($p > 0.05$) among mid-sized culture media, but varied between 16.35 and 20.67 μm among the treatments employed (Table 1).

Whereas total organic carbon was highest ($p < 0.01$) in NPK medium (> 16.04 pg cell^{-1}), in the case of media CHU_{12} and macrophyte + NPK, the highest organic carbon rate was obtained in the glass fiber recipient (Table 1).

Protein and fiber contents did not vary ($p > 0.05$) among the treatments. In fact, protein content remained over 26% dry weight, whereas fiber reached 5.23% dry weight. Lipids varied between 2.97 and 12% dry weight, although percentage was over 8.5% dry weight in NPK medium ($p < 0.05$). There was no variation ($p > 0.05$) in dry weight and ash contents among treatments (Table 1).

Nitrate, ammonia, total phosphorus and orthophosphate remained over 1 mg L^{-1} in NPK ($p < 0.01$). Phosphorus contents in NPK ($p < 0.01$) were higher than 4 mg L^{-1}, although in the other two media they were lower and varied between 1.8 and 2.7 mg L^{-1}.

In the culture media conductivity was also high (> 45 µS cm^{-1}) with no significant differences ($p > 0.05$) among treatments (Table 2).

Free CO_2 and bicarbonate concentrations in media ($p < 0.01$) were affected by pH, when pH was alkaline (> 8); free CO_2 was lower than 0.5 mg L^{-1}; rates of bicarbonate were between 11 and 134 mg L^{-1} (Table 2).

Chlorophyll-*a* concentrations were very high (> 482 µg L^{-1}) owing to high contents of nutrients in the medium and to the continuous light cycle (24 hours). The highest concentrations were observed in CHU_{12} culture media ($p < 0.01$). Constant air bubbling of medium had an oxygen rate over 7.3 mg L^{-1} ($p > 0.05$) (Table 2).

It should be underscored that there were no significant differences ($p > 0.05$) among the recipients for all physical, chemical and biological variables analyzed (Tables 1 and 2).

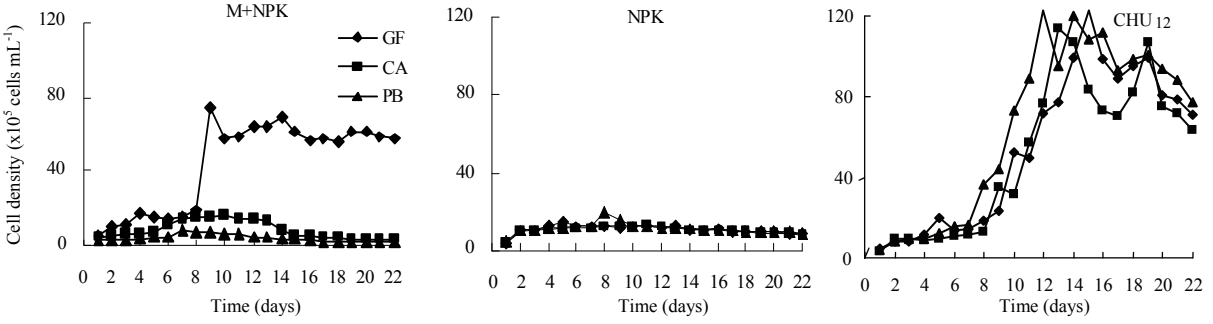

Figure 2. *Ankistrodesmus gracilis* growth curve cultured in macrophyte + NPK (M+ NPK), NPK and CHU_{12} media, in three different recipients, where: GF = glass fiber; CA = carboy; PB = plastic bag.

Table 1. Life history characteristics and biochemical composition of *Ankistrodesmus gracilis* algae cultured in macrophyte + NPK, NPK and CHU_{12} media, in three different recipients (glass fiber, carboy, and plastic bag), and results of two-way ANOVA between recipients and culture media, where: TOC = total organic carbon; DW= dry weight; $p > 0.05$ = non-significant; $p < 0.05$ and $p < 0.01$ = significant.

Characters	MACROPHYTE + NPK			NPK			CHU_{12}			ANOVA
	Glass fiber	Carboy	Plastic Bag	Glass fiber	Carboy	Plastic Bag	Glass fiber	Carboy	Plastic Bag	p > 0.05
Cell density (x 10^5 mL^{-1})	17.46 ± 2.60	13.61 ± 1.66	13.16 ± 1.42	12.18 ± 3.99	12.80 ± 3.46	11.52 ± 4.31	70.85 ± 33.20	62.92 ± 33.82	73.06 ± 35.07	p < 0.01
Growth rate (k)	0.30	0.14	0.16	0.38	0.24	0.46	0.40	0.42	0.44	p > 0.05
Doubling time (days)	3.28	7.08	6.42	2.66	4.20	2.16	2.51	2.36	2.28	p > 0.05
Mean cell size (μm)	19.78 ± 2.55	18.32 ± 2.07	16.35 ± 3.89	20.67 ± 3.06	20.60 ± 2.52	19.37 ± 3.52	17.72 ± 2.51	15.29 ± 2.68	17.65 ± 2.98	p > 0.05
Cell volume (μm^3)	92.56 ± 48.02	57.28 ± 28.25	60.75 ± 68.04	111.08 ± 62.62	104.72 ± 36.54	117.58 ± 93.78	92.31 ± 51.55	68.15 ± 35.02	89.88 ± 68.28	p < 0.01
TOC (pg cell^{-1})	14.13 ± 7.74	8.53 ± 4.42	9.18 ± 11.20	17.14 ± 10.17	16.04 ± 5.86	18.27 ± 15.62	14.11 ± 8.28	10.24 ± 5.54	13.78 ± 11.13	p < 0.01
Protein contents (% DW)	51.79 ± 0.47	56.53 ± 3.42	36.68 ± 0.12	46.53 ± 3.01	56.33 ± 1.92	63.99 ± 10.90	50.21 ± 5.21	38.76 ± 4.35	26.29 ± 0.01	p > 0.05
Lipids (% DW)	6.20 ± 0.29	8.59 ± 0.28	4.33 ± 0.04	10.00 ± 2.05	12.00 ± 10.57	8.54 ± 0.23	4.46 ± 0.00	2.97 ± 0.04	3.94 ± 0.52	p < 0.05
Fiber (% DW)	5.23 ± 1.54	10.67 ± 0.42	10.64 ± 0.04	10.40 ± 1.14	10.78 ± 8.89	22.34 ± 1.83	15.98 ± 2.17	13.48 ± 4.76	16.41 ± 8.21	p > 0.05
Ash contents (% DW)	3.89 ± 2.69	3.66 ± 2.15	1.93 ± 0.47	4.67 ± 2.30	3.58 ± 2.84	4.38 ± 1.79	3.95 ± 2.42	5.24 ± 2.73	2.70 ± 1.17	p > 0.05
Dry weight (pg cell^{-1})	4.82 ± 1.52	3.46 ± 3.47	4.76 ± 2.90	4.02 ± 3.17	3.26 ± 1.94	5.54 ± 2.68	3.05 ± 2.08	5.43 ± 2.63	4.35 ± 2.34	p > 0.05

Table 2. Mean variation of hydrological characteristics of the water culture of *Ankistrodesmus gracilis* algae during the experimental period, in three different recipients (glass fiber, carboy, and plastic bag) and media (macrophyte + NPK, NPK, and CHU_{12}), and results of two-way ANOVA between recipients and culture media, where: Temp.= temperature; Cond.= conductivity; DO = dissolved oxygen; Bicarb.= bicarbonate; CO_2= free CO_2; Ammon.= ammonia; TP= total phosphorus; Ortho.= orthophosphate; Chloro-*a*= chlorophyll-*a*; $p > 0.05$ = non-significant; $p < 0.05$ and $p < 0.01$ = significant.

Hydrological	MACROPHYTE + NPK			NPK			CHU_{12}			ANOVA
Characteristics	Glass fiber	Carboy	Plastic Bag	Glass fiber	Carboy	Plastic Bag	Glass fiber	Carboy	Plastic Bag	$p > 0.05$
Temp. (°C)	22.40 ± 1.29	22.57 ± 1.61	26.19 ± 1.05	22.9 ± 0.73	25.8 ± 0.38	22.7 ± 0.82	21.38 ± 0.87	22.57 ± 0.79	23.05 ± 0.95	$p > 0.05$
pH	7.31 ± 0.79	4.10 ± 1.28	4.47 ± 1.11	6.44 ± 0.21	6.21 ± 0.22	6.51 ± 0.14	8.69 ± 0.21	8.94 ± 0.41	9.11 ± 0.27	$p < 0.05$
Cond. (μS cm^{-1})	45.33 ± 7.67	95.71 ± 12.24	48.90 ± 13.51	92.0 ± 11.83	89.9 ± 10.1	82.57 ± 8.99	88.62 ± 7.60	89.24 ± 6.84	90.24 ± 6.95	$p > 0.05$
DO (mg L^{-1})	15.67 ± 1.16	9.69 ± 3.85	10.98 ± 5.58	7.30 ± 0.59	7.36 ± 0.60	8.02 ± 0.20	7.71 ± 0.82	7.77 ± 0.84	8.36 ± 0.37	$p > 0.05$
Bicarb. (mg L^{-1})	121.69 ± 22.24	114.66 ± 43.06	110.65 ± 45.48	63.87 ± 31.09	62.51 ± 27.64	52.25 ± 30.34	134.16 ± 96.63	131.96 ± 64.55	111.00 ± 97.31	$p < 0.01$
CO_2 (mg L^{-1})	55.22 ± 102.32	38.14 ± 93.64	45.59 ± 45.61	66.69 ± 58.74	62.29 ± 67.94	43.76 ± 08.11	0.38 ± 0.11	0.27 ± 0.15	0.19 ± 0.26	$p < 0.01$
Nitrate (μg L^{-1})	570.67 ± 211.93	613.26 ± 145.83	378.60 ± 110.77	1,622 ± 547.4	1,556 ± 806.6	1,814 ± 431.6	617.37 ± 445.45	799.91 ± 317.1	933.72 ± 37.18	$p < 0.01$
Nitrite (μg L^{-1})	136.34 ± 116.05	196.71 ± 170.53	182.53 ± 114.73	38.87 ± 67.94	9.53 ± 5.50	44.15 ± 19.56	233.58 ± 175.39	347.26 ± 125.7	389.11 ± 182.69	$p < 0.01$
Ammon. (μg L^{-1})	336.79± 191.51	244.04 ± 90.00	422.49 ± 191.92	3,986 ± 274.7	4,145 ± 337.6	3,953 ± 216.9	746.28 ± 403.82	513.33 ± 366.3	530.19 ± 573.43	$p < 0.01$
Ortho. (μg L^{-1})	603.39 ± 107.35	754.65 ± 219.22	772.15 ± 196.30	1,174 ± 161.86	1,082 ± 132.4	1,060 ± 166.3	2,425 ± 571.52	2,203 ± 688.39	1,772 ± 694.61	$p < 0.01$
TP (μg L^{-1})	2,536.51 ±695.25	2,739.78 ± 1.316.8	2,313.15 ± 793.71	4,123 ± 396.86	4,018 ± 254.4	4,188 ± 383.9	2,765 ± 788.54	2,264 ± 1.146	1,846 ± 460.22	$p < 0.01$
Chloro-*a* (mg L^{-1})	1,950 ± 780.02	1,040 ± 607.57	819.28 ± 618.02	1,120 ± 498.2	731.8 ± 282.6	482.0 ± 260.2	4,471 ± 1.548	3,404 ± 1.224	1,954 ± 872.57	$p < 0.01$

Discussion

The growth curve of *A. gracilis* in macrophyte + NPK medium, similar only in carboy and plastic bag recipients, was associated with acid pH (respectively 4.1 and 4.47). Certain Chlorophyceae algae fail to grow in acid pH. Consequently, algae activity decreases temporarily and recovers after several days (WIDJAJA et al., 2009).

Microalgae are sensitive to pH changes and its control is essential for keeping a high growth rate. As microalgae are able to metabolize the inorganic carbon CO_2, there is an equilibrium trend for pH increase (ROCHA et al., 2003).

The best cell yield was reported in medium CHU_{12}, since it contained all the essential macro- and micro-nutrients for alga development. Since traditional medium is very expensive for large scale culture, the use of alternative media is necessary in large scale alga production on condition that rapid growth occurs, as reported in macrophyte + NPK medium (8th day) previous to that of the commercial medium CHU_{12} (12th day), with high nutrition rate.

Growth rate is a function of total daily light input. The light period (24h) in the current study caused fast exponential growth. Further, macrophyte + NPK medium has a dark coloring, which not only hinders the penetration of light but also impairs growth above 15.5 x 10^5 cell mL^{-1}, after the exponential phase in carboy and plastic bag recipients, and keeps growth above 69.2 x 10^5 cell mL^{-1} in fiberglass recipients. Nevertheless, result rates were higher than those reported by Rodrigues and Belli-Filho (2004) for *Chlorella minutissima* cultured in a swine excrement medium (2.1 x 10^5 cell mL^{-1}) and by Sipaúba-Tavares and Pereira (2008) for *A. gracilis* cultured in NPK (135 x 10^4 cell mL^{-1}).

Light is one of the main factors that interfere in the growth of microalgae. Lin et al. (2007) show that an irradiance of 100 $\mu E\ m^{-2}\ s^{-1}$ for marine microalgae culture growth may provide optimal nutritional value for fish larvae. Irradiation conditions in the current analysis were lower, although the 24-h cycle was maintained. The cell number is highly dependent on culture conditions, namely lighting, culture media, CO_2 supply, cell age, etc.

The number of variables that affect microalgae growth rate is high, with direct, indirect and cross-effect influences. In fact, growth rate optimization is always a difficult task for each species (ROCHA et al., 2003).

Lighting (both natural and artificial) and temperature of microalgae cultures are very dependent variables. Mixing is the most practical way to dilute radiation evenly to all cells in the culture while improving the light regime. Microalgae do not distinguish between natural and artificial light, albeit more sensitive to light intensity and light/dark cycles (ROCHA et al., 2003).

Size and shape indicate available alga cell volume as food for the several organisms in the trophic chain (SIPAÚBA-TAVARES; ROCHA, 2001). Results show that alternative media used may be employed in the culture of *A. gracilis*, with the best results for cell volume in NPK medium.

The cell volumes obtained in the current research were higher than those reported by Sipaúba-Tavares and Rocha (1993) on the same alga (90 μm^3) and were lower than those reported by Hardy and Castro (2000) with *A. gracilis* grown in NPK medium (419.3 μm^3). In fact, the size and weight of each cell is not always the same (ROCHA et al., 2003).

Dry weight observed in this study was less than that reported by Hardy and Castro (2000) for *A. gracilis* in NPK medium (125.0 ± 13.1 pg $cell^{-1}$) and by Sipaúba-Tavares et al. (1999) in CHU_{12} medium (128.79 ± 8.8 pg $cell^{-1}$), cultivated in 2 L. The opposite has been reported for ash contents, which showed values over 1.93 pg $cell^{-1}$ dry weight, much higher than those reported by Sipaúba-Tavares and Rocha (1993) with the same alga in CHU_{12} medium (0.11 pg $cell^{-1}$ dry weight).

The mean size band in current research was lower than that reported by Sipaúba-Tavares and Rocha (1993) for *A. gracilis* in NPK medium (23.8 μm). According to these authors, this may be associated with differences in strains, culture conditions and volume of medium. According to Olivera and Sipaúba-Tavares (2000), the greater the water volume for alga culture, the higher the chance of a decrease in alga yield with regard to cell size. This is due to the competitiveness of algae for nutrients and light.

Carbon rate characterizes the alga nutritional value, normally between 40 and 60% dry weight, and evaluates food quality. Chlorophyceae algae have thin cell walls and thus a high organic carbon rate when compared to that of dry weight. Consequently, they are the best food for fresh water fish when compared to Diatomaceae, which have a basic role in sea environments (SIPAÚBA-TAVARES; ROCHA, 1993).

Results of organic carbon in the current research are better than those reported by Sipaúba-Tavares et al. (1999) for *A. gracilis* (2.6 pg $cell^{-1}$) cultivated in NPK medium, in a 2 L volume. This fact shows the possibility of macrophyte + NPK and NPK medium in plastic bags for the production of *A. gracilis* as live feed for fish larvae during their initial development phase.

Protein content above 30% is adequate and directly related to the aeration of the environment. As a rule, mean alga protein content ranges between 16 and 70% dry weight (BROWN et al., 2009). Protein contents of *A. gracilis* were over 26% dry weight, featuring more than 50% dry weight in NPK and macrophyte + NPK.

Complex B vitamins improve alga nutritional content, whereas the introduction of more vitamins from the first instances in the aquatic food chain increases vitamin content in animals reared in aquaculture systems (SOUTO et al., 2008).

High lipid contents (> 8.5% dry weight) in NPK for the three recipients coincided with high levels of phosphorus (> 4.0 mg L^{-1}), ammonia (> 3.9 mg L^{-1}) and nitrate (> 1.5 mg L^{-1}). These numbers disagree with results by Widjaja et al. (2009) who reported high lipid contents for the green alga *Chlorella vulgaris* when a decrease in nitrogen in the medium occurred. In the current study, high lipid content was directly associated with high CO_2 levels in NPK. This is due to the fact that when the compound had lipid content over 62 mg L^{-1}, it reached 10% dry weight (glass fiber and carboy). A CO_2 decrease in the plastic bag recipient coincided with lower lipid content (8.5% dry weight) in NPK. According to Widjaja et al. (2009), there is a direct co-relationship between CO_2 increase in culture medium and lipid increase in algae cells.

Ammonia concentrations above 1 mg L^{-1} in culture media may affect the development of *A. gracilis* which usually inhibit biomass growth (SIPAÚBA-TAVARES; PEREIRA, 2008). This factor may have been the cause of lesser densities in NPK (< 17.5 x 10^5 cell mL^{-1}) when compared to those in CHU_{12}.

High chlorophyll-*a* contents (> 480 mg L^{-1}) are associated with the intrinsic conditions of alga of the Chlorophyceae class, which has large amounts of chlorophyll, or rather, approximately 1 to 45% dry weight (CHEN et al., 2009).

The plastic bag for small and large-scale culture of *A. gracilis* is feasible due to its performance in alga morphological characteristics, but also due to its costs. The cost of each unit is US$ 3.96, extremely low when compared to the glass fiber with a unit cost of US$ 162.96. Since the carboy recipient had the highest cost, approximately US$ 1,138.92 for 13 L, it turned out to be unworkable for large-scale culture. Further, if the low cost plastic bag is well utilized, it may be used three or four times consecutively, and thus a gain in production ensues.

Conclusion

NPK or even macrophyte associated with NPK is an alternative medium that may be used for *A. gracilis* culture or for other Chlorophyceae algae

with a higher or similar nutritional rate to that of the traditional one (CHU_{12}). No significant difference ($p > 0.05$) in the biology of *A. gracilis* was reported among recipients. The plastic bag may be another alternative to be employed with good growth rates, low costs and smaller culture spaces in the laboratory. Other studies, mainly for the improvement of *A. gracilis* growth, may be undertaken with regard to pH of the medium and high contents of nutrients, so that mass production technology could be obtained while employing low cost media and recipients, coupled with high quality.

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