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Development of low-cost culture media for *Ankistrodesmus gracilis* based on inorganic fertilizer and macrophyte

Desenvolvimento de meios de cultura de baixo custo para *Ankistrodesmus gracilis* à base de fertilizante inorgânico e macrófita

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Abstract: Aim: The influence of three different culture media on the growth and biochemical composition of *Ankistrodemsus gracilis* microalgae is evaluated. **Methods:** Microalgae were placed in a bath culture during 28 days, with inorganic fertilizer (NPK) and macrophyte *Eichhornia crassipes* as culture medium to compare the growth of microalgae and commercial medium CHU_{12}. Protein and lipid contents, water conditions of the culture medium and physiological parameters were assessed weekly. **Results:** Growth rate, macro-minerals, micro-nutrients and nutritional values were similar to commercial culture medium in the media with inorganic fertilizer and macrophyte. The biological indexes of microalgae were not influenced by weekly differences during the study period. Although lipid contents, nitrogen and growth rate of *A. gracilis* were higher in inorganic fertilizer and macrophyte media; Mn, B, Cu, S, dry matter, ash content, cell volume, TOC and total length were similar among the media used. The same high cell density 25 x 10^5 cells.mL^{-1} was obtained in NPK and in CHU_{12} medium respectively after 11 and 14 days cultivation. **Conclusion:** Culture media inorganic fertilizer (NPK) and macrophyte (*Eichhornia crassipes*), were adequate and may replace the commercial medium CHU_{12} for the cultivation of microalgae *A. gracilis*, resulting in high nutritional composition, high biomass, high nutrients and low-cost.

Keywords: *Eichhornia crassipes*; NPK (20-5-20); biochemical composition; culture media.

Resumo: Objetivos: Este estudo avaliou a influência de três diferentes meios de cultura para o crescimento e composição bioquímica da microalga *Ankistrodesmus gracilis*. **Métodos:** A microalga foi cultivada em sistema estático durante 28 dias utilizando o fertilizante inorgânico (NPK) e a macrófita *Eichhornia crassipes* como meio de cultura, comparando o desempenho desta microalga com o meio comercial CHU_{12}. Os teores de proteína e lipídios, condições da água do meio de cultura e variáveis fisiológicas foram avaliados semanalmente. **Resultados:** Os meios com fertilizante inorgânico e com macrófita apresentaram condições de crescimento e valor nutricional similar ao meio de cultura comercial. Os índices biológicos da microalga não foram influenciados por diferenças semanais durante o período do estudo. O teor de lipídio, nitrogênio e a taxa de crescimento de *A. gracilis* foram superiores no meio com fertilizante inorgânico e com macrófitas. Já o teor de Mn, B, Cu, S, massa seca, teores de cinzas, o volume celular, TOC e comprimento total foram semelhantes nos meios utilizados. As maiores densidades celulares de 25 x 10^5 células.mL^{-1} foram obtidas no meio NPK com 11 dias de cultivo e no meio CHU_{12} com 14 dias de cultivo. **Conclusão:** Os meios de cultura

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com fertilizante inorgânico (NPK) e com macrófita (*Eichhornia crassipes*) foram adequados e podem substituir o meio comercial CHU_{12} para o cultivo da microalga *A. gracilis* tendo como resultados elevados valores nutricionais, elevada biomassa, altos teores de nutrientes com baixo custo.

Palavras-chave: *Eichhornia crassipes*; NPK (20-5-20); composição bioquimíca; meio de cultura.

1. Introduction

Several studies on microalgae biology have been recently undertaken with microorganisms as a protein source, mainly in the aquaculture industry, as feed for fish larvae or with by-products such as lipids, carbohydrates, vitamins, minerals and pigmentation (keto-carotenoid astaxanthin). The cultivation of microalgae as prime feed for fish larvae is currently regarded as essential in aquaculture study albeit expensive as a live food source. A possible solution to minimize the, high costs of microalgae cultivation is the use of an alternative medium to obtain microalgae biomass, which is essential as live food for fish larvae. The production of microalgae is a high economical investment due to the high costs of the culture medium to obtain microalgae biomass.

Strategies such as alternative media should be undertaken to decrease costs without affecting the nutritional values of microalgae. Successful attempts have been made to substitute commercial media for alternative, less expensive ones. For instance, an inorganic fertilizer is feasible since it is widely available, it dissolves easily, it has a well-defined composition, or rather high nitrogen and phosphorus contents, and triggers moderate pH in the medium (Tew et al., 2006). Microalgae grow in an inorganic fertilizer (NPK, 20-5-20) which has a similar or greater effect on the growth of microalgae than commercial medium (Ashraf et al., 2011; Brito et al., 2013; Sipaúba-Tavares et al., 2011). Some authors have indicated that the agricultural fertilizer media were eight times cheaper than conventional medium (Ashraf et al., 2011; El-Nabris, 2012; Brito et al., 2013).

Another alternative medium for the growing of microalgae consists of a mixture of macrophytes (*Eichhornia crassipes*) and inorganic fertilizers (NPK). In fact, the aquatic plant has a high concentration of nitrogen and phosphorus and other nutrients which are important for the growth of microalgae at low costs (Sipaúba-Tavares et al., 2009).

The concentration of nitrogen and phosphorus in culture media has a fundamental and direct influence on microalgae growth, besides being closely related to lipid accumulation (Lee et al., 2013). Growth rate, cell density and lipid contents of microalgae directly contribute towards the biological indexes of the microorganisms (Huang et al., 2013). Increase in lipid accumulation is related to an increase in iron concentration in the medium. Liu et al. (2008) observed that *Chlorella vulgaris* showed high lipid accumulation when there were high iron concentrations in the medium. However, other important micronutrients, such as boron, copper, zinc and manganese albeit in smaller amounts, are required, for the growth of microalgae (Larsdotter, 2006).

Culture medium conditions such as temperature, light, pH and nutrients, affect not only photosynthesis and productivity of microalgae cells but also influence the activity of cell metabolism and its composition (Huang et al., 2013).

Microalgae, such as *Ankistrodesmus gracilis*, have been used in aquaculture due to their rapid growth rate, resistance to adverse conditions, nutritional quality and also as model organism for the analysis of cell growth and division. Detailed knowledge on microalgae culture in alternative media and at low costs for the cultivation of *A. gracilis* may be efficient at least in batch culture mode to improve physiological difference more than the commercial media. Current assay investigates weekly biological performance of *Ankistrodesmus gracilis* for a low-cost culture media based on the use of an inorganic fertilizer (NPK) and macrophyte (*Eichhornia crassipes*).

2. Methods

2.1. Microalgae cultures and laboratory conditions

Current study on *A. gracilis* comprised three trials namely, microalgae cultivated in alternative media, NPK (20-5-20), macrophyte with NPK (M+NPK), and in pure commercial medium (CHU_{12}) as control, and cells grown in batch culture. The *A. gracilis* strain used in current study was obtained from culture collection number 005CH, originally from Broa Reservoir (22º15' S and 47º19' W), Brazil. Algae were batch cultured at 22 ± 2 °C and, exposed to light at 40 $\mu mol.m^{-2}.s^{-1}$, while culture parameters were measured weekly during the 28 day experiment. The growth of *A. gracilis* involved inorganic fertilizer NPK

medium (20-5-20) (Sipaúba-Tavares & Rocha, 1993), commercial CHU_{12} medium and medium with a mixture of macrophyte (*Eichhornia crassipes*) extract and NPK (20-5-20) (Sipaúba-Tavares et al., 2009). The mixture contained approximately 5 kg of *Eichhornia crassipes*, which was ground and boiled in distilled water for one hour. The hot extract was filtered and autoclaved at 120 °C, during 20 minutes. After autoclaving, a sample (70 mL) was collected and, after cooling it was diluted with distilled water up to 1.4-L when 2.5 mL NPK were added (Sipaúba-Tavares et al., 2009). The experiment started with 250 mL flasks, with microalgae density of 2 x 10^5 cells.mL^{-1} and cultured in NPK medium. When cultures reached the late exponential growth phase (7 day), about 200 mL of culture density (0.7 x 10^5 cells.mL^{-1}) were added in 2-L flasks with NPK medium. After a 7 day exponential growth phase, the culture was transferred to sterilized recipients containing 13-L at density 4.4 x 10^5 cells.mL^{-1} of NPK medium; 5.1 x 10^5 cells.mL^{-1} of macrophyte with NPK medium (M+NPK); and 4.4 x 10^5 cells.mL^{-1} of CHU_{12} medium. The experiments were performed in 13-L volumes with continuous air bubbling. Vitamin B_{12} complex was added to NPK and macrophyte with NPK medium at the rate of 0.02 g.L^{-1} plus biotin (0.01 mg.L^{-1}) (Table 1). Growth performance and other physiological parameters and analytical method were analysed weekly (1, 7, 14, 21, 28 days) during the study period.

2.2. Growth performance

Triplicate 1 mL aliquots were removed daily from the microalgae culture and a minimum of 2 x 1 μL sub-samples were used for cell quantification by a Neubauer hemocytometer. Growth rate (k) was calculated by the formula: $k = (3.322/t_2 - t_1 \times \log N_2/N_1)$ (t = time; N = number of cells; subscripts denote values at different times) (Guillard, 1973). Division per day was calculated by formula: $Div.day^{-1} = K/\ln_2$ (Foggs & Thake, 1987). Doubling time (cell division time or generation time) was calculated from results obtained from growth rate, by the formula: $Td = 1k^{-1}$ (Td = duplication time, $1k^{-1}$ = days per division) (Guillard, 1973). Dry biomass and ash content were determined following Vollenweider (1974). Total length of 50 specimens was determined with microscope Leica DFC 295 by image analysis system LAS core (LAS V3.8), with a 40X micrometric objective. Cell volume was calculated by mean cell size with the use of the most appropriate geometric form, or rather the sphere formula (Hillebrand et al., 1999). Total organic carbon (TOC) was calculated by $C = 0.1204.V^{1.051}$ (C = carbon content in pg.$cell^{-1}$; V = cell volume) using regression according to Rocha & Duncan (1985).

2.3. Microalgae biomass and parameters of culture media

The physical and chemical parameters of the culture media and microalgae biomass were performed weekly. Dissolved oxygen, pH and conductivity of culture media were measured with multiparametric probe YSI 556 MPS. Total ammonia nitrogen (TAN) was quantified with a spectrophotometer according to Koroleff (1976) and total phosphorus was quantified as described by Golterman et al. (1978). Chlorophyll-*a*, concentration was determined by colorimetric analysis using spectrophotometer after extraction with 90% ethanol (Nusch, 1980). The biomass of microalgae was harvested, centrifuged, and lyophilized for the analysis of proteins, lipids (AOAC, 1990) and macro-minerals (N, P, K, Ca, Mg and S) and micro-nutrients (B, Cu, Fe, Mn, and

Table 1. Composition of nutrients (g.L^{-1}) of different culture media: NPK; Macrophyte with NPK (M+NPK) and CHU_{12}.

Ingredients	Medium		
	NPK	M+NPK	CHU_{12}
P_2O_5	2.5	-	-
K_2O	10	-	-
N	10	0.095	-
P	-	0.091	-
C	-	0.199	-
Mn	-	0.06	-
Mg	-	0.003	-
Fe	-	2.18	-
K	-	0.056	-
Ca	-	0.001	-
Cu (mg)	-	0.05	-
Thiamine	0.007	0.007	-
Vit B_2	0.007	0.007	-
Vit B_6	0.005	0.005	-
Vit B_{12} (μg.L^{-1})	33	33	-
Biotin (mg.L^{-1})	0.01	0.01	-
$Ca(NO_3)_2$	-	-	3.0
Na_2CO_3	-	-	2.0
KCl	-	-	0.5
K_2SiO	-	-	0.5
$MgSO_47H_2O$	-	-	7.5
K_2HPO_4	-	-	0.5
$FeCl_3.6H_2O$	-	-	0.5

Zn) of microalgae biomass was performed according to the methods describe by Bataglia et al. (1983).

2.4. Analysis of cost culture medium

The costs of culture media components include the prices of vitamin B complex, NPK fertilizer and different raw materials for commercial medium (CHU_{12}). The costs analysed for a 2-L producing 100,000L month^{-1} (g.L^{-1} dry mass) of culture medium.

2.5. Data analysis

All data were subjected to one-way analysis of variance (ANOVA) with Statistica 8.0, to test the effects of the medium. Differences were significant at $p<0.05$. All results were expressed as means ± SD (Standard Deviation). Statistical analyses were performed with Statistica 8.0 (STATSOFT, 2007) and experiments were carried out in triplicate, except protein and lipid that were done once a week.

3. Results

After the 28 day experimental period, the weekly performance of *A. gracilis* microalgae revealed that cell growth was different during the log phase (1-11 days for NPK; 1-14 days for M+NPK; 1-15 days for CHU_{12}). The highest cell density was observed for the NPK medium (11-days) and commercial medium CHU_{12} (14-days) with 25.5 x 10^5 cells.mL^{-1} and M+NPK medium (13-days) with 24.2 x 10^5 cells.mL^{-1}. *Ankistrodesmus gracilis* microalga grown on CHU_{12} medium showed lower cell density during senescent phase (16-28 days), ranging between 12.1 and 22.3 cells.mL^{-1}. In NPK medium, density was highest during the log phase (1-11 days) that ranged between 5.4 and 25.5 cells.mL^{-1}. *Ankistrodesmus gracilis* microalga cell density decreased in all media from the 13th day (M+NPK and NPK) and 15th day (CHU_{12}). *Ankistrodesmus. gracilis* microalga cell density in the three culture media varied between 4.3 x 10^5 cells.mL^{-1} and 25.6 x 10^5 cells.mL^{-1} in CHU_{12} medium; between 3.2 x 10^5 cells.mL^{-1} and 24.2 x 10^5 cells.mL^{-1} in M+NPK medium and between 5.4 x 10^5 cells.mL^{-1} and 25.5 x 10^5 cells.mL^{-1} in NPK medium (Figure 1).

The growth rate (k=0.24) and division day (0.35 div. day^{-1}) were higher in M+NPK medium. Chlorophyll-*a* and doubling time were higher ($p<0.01$) in CHU_{12} medium. Cell volume, dry biomass, total length, ash content and TOC were similar ($p>0.05$) in the culture media. *Ankistrodesmus gracilis* microalgae in inorganic fertilizer and macrophyte culture media showed higher biological indexes such as growth rate and division per day (Table 2). Microalgae protein content ranged between 27% and 48% of total algal dry weight and lipids contents below 14% of dry weight. As a rule, protein and lipid contents

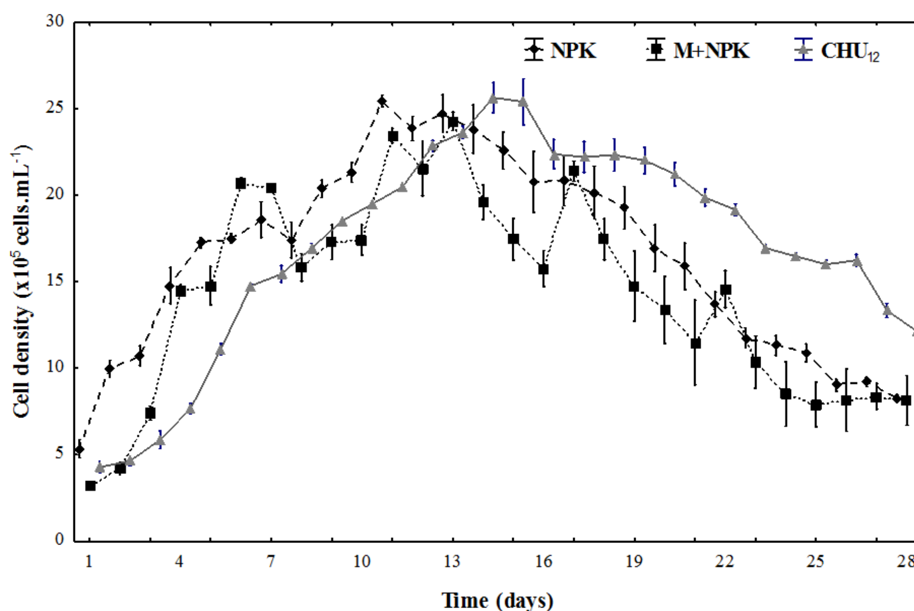

Figure 1. Growth of *Ankistrodesmus gracilis* in three different media: inorganic fertilizer (NPK), macrophyte with inorganic fertilizer (M+NPK) and commercial (CHU_{12}). Data represent mean ± SD of three replicates per medium (NPK, M+NPK and CHU_{12}).

were higher in NPK and M+NPK media than in commercial medium (Table 2).

Lower dissolved oxygen concentrations (5.0-5.5 $mg.L^{-1}$) and conductivity (320-470 $\mu S.cm^{-1}$) were registered in M+NPK medium, but higher pH (7.9-10) occurred in CHU_{12} and M+NPK culture media. Decrease in total ammonia nitrogen and total phosphorus in M+NPK medium on the 21st day was observed when pH ≤ 5 a sharp protein decrease occurred (Table 2, Figures 2 and 3). Higher total phosphorus (4.1-5.0 $mg.L^{-1}$) was obtained in NPK medium and TAN was higher in

Table 2. Parameters of *Ankistrodesmus gracilis* cultured in inorganic fertilizer (NPK), macrophyte with inorganic fertilizer (M+NPK) and commercial media (CHU_{12}). Data represent mean ± SD* of three replicates per medium (NPK, M+NPK and CHU_{12}).

Parameters	Medium		
	NPK	M+NPK	CHU_{12}
Chlorophyll-*a* ($mg.L^{-1}$)	758 ± 217[b]	357 ± 166[c]	1,899 ± 821[a]
Mean cell density ($x10^5$ cell mL^{-1})	16.2 ± 6[a]	12.7 ± 5[b]	16.5 ± 6[a]
Growth rate (k)	0.22	0.24	0.19
$Division.day^{-1}$ (div. day^{-1})	0.32	0.35	0.32
Doubling time (days)	4.47	4.81	5.04
Dry weight (pg. $cell^{-1}$)	5.1 ± 3[a]	4.4 ± 2[a]	3.5 ± 2[a]
Ash (% dry biomass)	1.5 ± 0.3[a]	2.7 ± 1.7[a]	1.7 ± 0.6[a]
Protein (% dry biomass)	41.3 ± 3.4[a]	37.7 ± 7.6[a]	31.8 ± 4.8[b]
Lipid (% dry biomass)	12.3 ± 0.8[a]	9.9 ± 1.3[b]	2.8 ± 1.4[c]
Total lenght (µm)	14.2 ± 1[a]	14.7 ±1[a]	13.4 ± 1[a]
Cell volume (μm^3)	65.7 ± 16.2[a]	71.2 ± 20.1[a]	59.8 ± 11.2[a]
TOC ($pg.cell^{-1}$)	9.9 ± 2[a]	10.8 ± 3[a]	8.9 ± 2[a]
Conductivity ($\mu S.cm^{-1}$)	1,144 ± 23[a]	1,125 ± 40[a]	384 ± 55[b]

*In each row, means followed by the same letter do not significantly differ ($p < 0.05$).

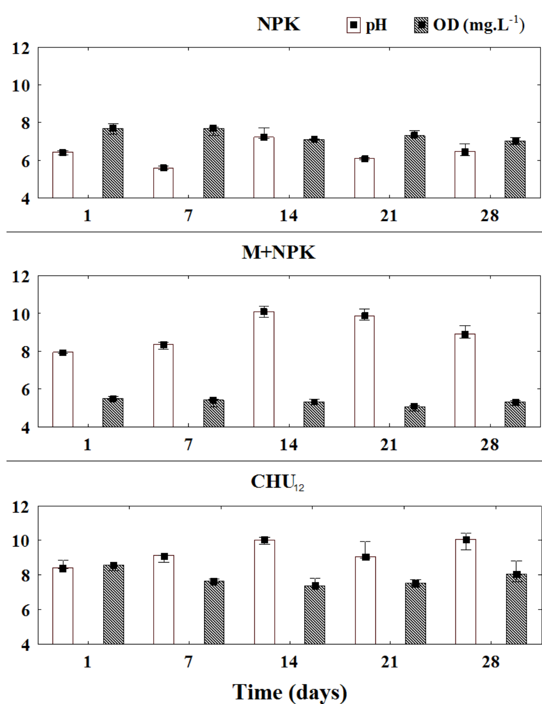

Figure 2. Weekly variations of pH and dissolved oxygen (DO) of culture media parameters from three different media: inorganic fertilizer (NPK), macrophyte with inorganic fertilizer (M+NPK) and commercial (CHU_{12}). Data represent mean ± SD of three replicates per medium (NPK, M+NPK and CHU_{12}).

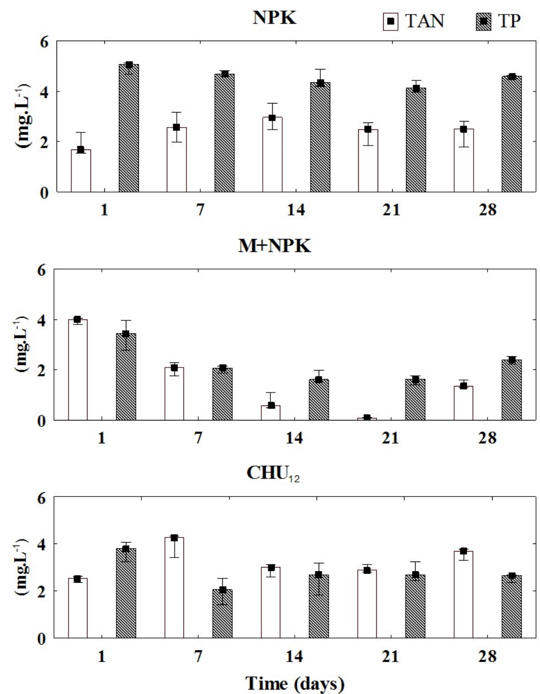

Figure 3. Weekly variations of total phosphorus (TP) and total ammonia nitrogen (TAN) of culture media parameters from three different media: inorganic fertilizer (NPK), macrophyte with inorganic fertilizer (M+NPK) and commercial (CHU_{12}). Data represent mean ± SD of three replicates per medium (NPK, M+NPK and CHU_{12}).

CHU_{12} (2.9-4.0 g.L^{-1}) (Figure 3). Weekly variations ($p<0.05$) were observed in microalgae biomass of culture media parameters during the study period (28 days). Difference ($p<0.05$) was observed in total phosphorus, total ammonia nitrogen, pH and conductivity in the culture media (Figure 3).

Biomass of *A. gracilis* microalgae demonstrated high levels of nutrients in NPK and CHU_{12} media. Although the commercial medium CHU_{12} had all the nutrients necessary for microalgae growth, NPK and M+NPK media for *A. gracilis* microalgae biomass showed higher levels of the elements under analysis. Sulfur, B, Cu and Mn were similar ($p>0.05$) among the analysed minerals in the three culture media, although nitrogen was higher in inorganic fertilizer and macrophyte media. Potassium and Fe were higher in NPK medium, whilst P, C and Mg were higher in CHU_{12} medium. Although zinc was lower in M+NPK medium, the element was similar in the others culture media (Table 3). NPK and M+NPK culture media registered a 69% and 94% cost reduction, respectively, when compared to commercial medium (CHU_{12}). Estimation of yield of microalgae biomass were 3.9 g.L^{-1} for M+NPK, 3.1 g.L^{-1} for CHU_{12} and 4 g.L^{-1} for NPK of dry biomass of *A. gracilis*, ranged cost between US$6,600 (M+NPK) and US$22,200 (CHU_{12}), while the average cost per liter was US$0.11 for M+NPK, US$0.57 for NPK and US$1.89 for CHU_{12} (Table 4).

4. Discussion

Microalgae in NPK and M+NPK culture media had a similar productive response to that reported in commercial medium. The growth rate was higher in NPK and M+NPK media than in commercial medium due to the combination of phosphorus, nitrogen and potassium contents; which always produced higher algal densities. Ashraf et al. (2011) concluded that phosphorus was one of the limiting nutrients for *Chlorella vulgaris* growth. According to Shilton et al. (2012), phosphorus removal in algae is due to biomass growth and phosphorus content in the macrophytes and algal biomass may be tripled from 1% to 3% via luxury uptake. In the NPK

Table 3. Values obtained for macronutrients and micronutrients (g.L^{-1}) of *Ankistrodesmus gracilis* biomass in inorganic fertilizer (NPK), macrophyte with inorganic fertilizer (M+NPK) and commercial medium (CHU_{12}). Data represent mean ± SD* of three replicates per medium (NPK, M+NPK and CHU_{12}).

Nutrient Composition	Media		
	NPK	M + NPK	CHU_{12}
Boron (B)	6 ± 4[a]	3 ± 1[a]	7 ± 1[a]
Calcium (Ca)	12 ± 11[b]	6 ± 3[b]	66 ± 31[a]
Copper (Cu)	52 ± 20[a]	25 ± 27[a]	21 ± 16[a]
Iron (Fe)	3,992 ± 1844[a]	1,151 ± 1143[b]	2,922 ± 1540[ab]
Magnesium (Mg)	3 ± 1[b]	2 ± 1[b]	28 ± 7[a]
Manganese (Mn)	112 ± 99[a]	54 ± 23[a]	65 ± 23[a]
Nitrogen (N)	60 ± 7[a]	54 ± 4[ab]	46 ± 8[b]
Phosphorus (P)	16 ± 7[ab]	8 ± 2[b]	22 ± 4[a]
Potassium (K)	9 ± 2[a]	7 ± 1[ab]	6 ± 1[b]
Sulfur (S)	6 ± 1[a]	4 ± 2[a]	5 ± 1[a]
Zinc (Zn)	75 ± 24[a]	35 ± 17[b]	67 ± 12[a]

*In each row, means followed by the same letter do not significantly differ ($p < 0.05$).

Table 4. Estimation of components cost (US$ dollars)[a] for 2-L producing 100,000L month[1] (g.L^{-1} dry biomass) of *Ankistrodesmus gracilis* in different culture media (NPK, M+NPK and CHU_{12}).

Production Input	Culture Media		
	NPK	M+NPK	CHU_{12}
Costs (US$)			
Cost per liter of concentrated medium	0.57	0.11	1.85
Total annual costs*	19,542	6,600	22,200
Production			
Yield of one liter the concentrated culture medium (L^{-1})	35	20	100
Yield of microalgae biomass (g.L^{-1} dry biomass)	4.0	3.9	3.1
Annual biomass production (tonne dry biomass)*	4,800	4,680	3,722

[a]Average exchange rate (February 2017) US$1.00 = R$ 3.11; *Estimate based on a production of 100,000 liters per month.

medium, the phosphorus in *A. gracilis* biomass was similar to commercial medium (CHU_{12}).

Ankistrodesmus gracilis grown in alternative media reached cell density close to 25.5 x 10^5 cells.mL^{-1} in NPK medium and 24.2 x 10^5 cells.mL^{-1} in M+NPK medium. These results were lower than those by Mártinez-Córdova et al. (2012) in their research on *Chaetoceros muller* with agricultural fertilizer, which reached 3.7 x 10^6 cells.mL^{-1} and higher than values by Kalita et al. (2011) which reached 9.0 x 10^5 cells.mL^{-1} in BG-11 medium with the same microalgae. The relative growth rate of microalgae is considerably affected by initial cell density and longer lag phase. The above factors may have influenced the growth of *A. gracilis* in CHU_{12} medium in current study. Secondary metabolites produced by microalgae are highly abundant in the stationary phase or in slower growing culture (Grabski & Tukaj, 2008). The same has been reported in current analysis where nutrients and the biochemical composition of *A. gracilis* microalgae were observed in this phase with the exception of nitrogen and protein in CHU_{12} medium with higher levels in the senescent phase. According to Grabski & Tukaj (2008), cell bio-volume, dry biomass content and photosynthesis demonstrated that results of growth processes of cell cycles depended on the medium. In current experiment the high density of *A. gracilis* was best in NPK and M+NPK culture media during the lag phase of growth, but the density of these microalgae after 13 days was better in the CHU_{12} medium.

Contents of lipids and proteins production in alternative media were somewhat higher than those obtained from microalgae in CHU_{12} medium. Lipid contents in current study were lower and varied from 5% in CHU_{12} medium to 15% in NPK medium, when compared to those by George et al. (2014) in cultures of *Ankistrodesmus falcatus* in BG-11 medium (67%). According to these authors, lipid production was best under light intensity 60 μmol.m^{-2}.s^{-1} for *A. falcatus*, whereas the microalga *A. gracilis* in current study was cultured under 40 μmol.m^{-2}.s^{-1} in all culture media. Efficient use of photons is one of the major factors that affect biomass and lipid production in microalga cultures (George et al., 2014). However, the biochemical composition of microalgae may be altered by changing the physical and chemical parameters of the culture medium (Huang et al., 2013).

Nitrogen contents in CHU_{12} and NPK culture media of current analysis were higher between 1 mg.L^{-1} and 4 mg.L^{-1}. High nitrogen conditions significantly decrease the lipid fraction of many microalgae and enhance the increase of protein levels (Uslu et al., 2011). Results in current assay were similar to those obtained by these authors. Although nitrogen sources responded well and enhanced microalgae growth, Arumugam et al. (2013) reported that maximum growth was recorded for potassium nitrate, indicating favorable nitrogen source for the growth of *Scenedesmus* sp. In fact nitrogen and potassium are actually two important nutrients for microalgae growth.

The addition of Fe, Mn, Mo and Ni in the culture medium for the growth of microalgae is extremely useful to assess the complementation to growth and lipid production (Song et al., 2012). Iron and Mn in the microalgae biomass were relevant to improve growth but not efficient to lipid accumulation, since nutrients were not high in the culture media, albeit important to fulfill the microalgae function in CHU_{12} and in M+NPK media. The effect of culture medium may also be perceived on the composition of microalgae. The presence of Zn, Mg, Fe, Cu and B are essential for photosynthesis and the metabolites of these micronutrients and are closely linked to protein synthesis and energy production (Hänsch & Mendel, 2009).

Current study indicated higher NPK and M+NPK media, including vitamin B_{12} complex, in term of money saving with commercial CHU_{12} medium. A great difference in price was found between CHU_{12} and M+NPK media, and microalgae culture in NPK and M+NPK media had a similar productive response to cultures in commercial medium. Annual biomass production was higher in NPK and M+NPK culture media and the cost per liter of commercial medium was higher than alternative media. Consequently, the use of inorganic fertilizer and *E. crassipes* with NPK may provide similar or better results at lower costs.

When all the factors mentioned above are taken into account, it may be concluded that biological indexes, growth performance and microalgae biomass were not influenced by weekly differences during the study period. Results showed that the NPK and M+NPK media was a good tool for the bath culture mode for *A. gracilis* in the laboratory. Further, protein content, lipids contents, N, growth rate and division per day in microalgae biomass were higher in NPK and macrophyte media. However, Mn, B, Cu, S, dry weight, ash contents, cell volume, TOC and total length were similar for all media.

Results showed NPK and macrophyte media had similar responses to biomass and nutritional values

and may indicate that combination of inorganic fertilizer and macrophyte supported the growth of *Ankistrodesmus gracilis* and confirmed adequate media to replace the commercial one (CHU_{12}). It would represent an alternative for culture media supplementation to *A. gracilis* to obtain a biomass with great impact from the economical point of view and feasibility. In commercial aquaculture, microalgae culture at low costs and high nutritional value are important to increase fish survival rate, mainly during the first days of live when live food is the basic feed sources.

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