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CUSTO DE DIFERENTES FONTES DE NITROGÊNIO EM CULTIVO DE MICROALGAS *Nannchloropsis oculata* E *Conticribra weissflogii* COM MEIO FERTILIZANTE

COST OF DIFFERENT NITROGEN SOURCES IN MICROALGAE Nannchloropsis oculata AND Conticribra weissflogii CULTURE WITH AGRICULTURAL FERTILIZERS

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Resumo: Os fertilizantes agrícolas são utilizados como nutriente para a produção massiva de microalgas. Vários meios de cultura utilizam amônio e uréia como fonte de nitrogênio. No entanto, análises de nutrientes indicam que as formas de nitrogênio não são igualmente utilizadas pelas diferentes espécies de microalgas, o que significa que o uso simultâneo de ambas as fontes de nitrogênio pode representar custos extras na produção. Neste estudo avaliamos qual forma de nitrogênio (amônio ou uréia) é preferencialmente assimilada pelas microalgas marinhas *Nannochloropsis oculata* e *Conticribra (Thalassiosira) weissflogii*. Os experimentos foram realizados com três tratamentos: a) Controle - com uréia e sulfato de amônio, b) Amônio - apenas com sulfato de amônio c) Uréia - apenas com uréia. Não houve diferença significativa na densidade celular entre os tratamentos com sulfato de amônio apenas e com ambas as fontes de nitrogênio (Controle) para as duas espécies. Esses dados sugerem que a remoção de uréia na produção em larga escala de microalgas não afeta o crescimento da espécie, e representa uma economia de 58% no preparo da solução de meio de crescimento.
Palavras-chave: microalga; meio de cultivo; fonte de nitrogênio; produção de biomassa.

Abstract: Agricultural fertilizers are used as nutrient for massive microalgae production. Several culture media utilize both ammonium and urea as nitrogen source. However, nutrient measurements indicate that both nitrogen species are not equally used by different microalgae species, meaning that simultaneous use of both nitrogen sources may represent extra costs in the microalgae production. In this study we evaluated which form of nitrogen (ammonium or urea) is preferentially assimilated by the marine microalgae *Nannochloropsis oculata* and *Conticribra (Thalassiosira) weissflogii*. Experiments were performed with three treatments: a) Control - with urea and ammonium sulfate, b) Ammonium -with only ammonium sulfate and c) Urea - with only urea. There was no significant difference in cell density between treatments with ammonium sulphate only and with both nitrogen sources (Control) for the two species. These data suggest that removal of urea in the large scale production of microalgae does not affect the growth of the species, and represent savings of 58% in the preparation of growth media solution.
Keywords: microalgae; culture media; nitrogen source; biomass production.

1 INTRODUCTION

Microalgae bioproducts are used by different industries as cosmetics, medicine, and bio-energy (KHAN *et al*., 2018; DE SOUZA *et al*., 2019). However, the success of commercial microalgae production depends on several factors, as well as to find the best cost-benefit relationship for all steps of the large-scale production (RAWAT *et al*., 2013, BARSANTI; GUALTIERI, 2018). The choice of the culture medium becomes more critical in large scale than in the laboratory, where the cultures are carried out in small volumes. Studies tried to determine the culture medium favorable to stimulate the growth of the species of interest without overly production costs (LOURENÇO, 2006; ASHOUR; EL-WAHAB, 2017; COVELL *et al*., 2020).

Culture medium with more than one nitrogen source is used in aquaculture for mass production of microalgae (PALANICHAMY; RANI, 2004; NETO *et al*., 2018). The use of both nutrients is to attend the necessities of different algal species that may exploit each one or both sources with varying levels, so that different nitrogen substrates may selectively stimulate the development of algal specie (BERMAN; CHAVA, 1999; ERRAT *et al*., 2020; KUMAR; BERA, 2020).

Ancillary tests conducted in our microalgae production demonstrated that large amounts of ammonium and urea remained in the culture medium even after the production of large quantities of microalgae biomass. This is a clear indication that the use of both nitrogen sources is probably unnecessary to guarantee the microalgae growth.

In order to test this hypothesis, we conducted a series of experiments with the marine microalgaes *Nannochloropsis oculata* (Droop) Hibberd DJ and *Conticribra weissflogii* (Grunow) K.Stachura-Suchoples & DM Williams (previously *Thalassiosira weissflogii*) to determine which form of nitrogen (ammonium or urea) is preferentially assimilated by these species.

These species were selected because they are used as food source in aquaculture (DA SILVA *et al*., 2013; KAPARAPU, 2018; TUGIYONO *et al*. 2018) they have excellent results in laboratory studies regarding the absorption of atmospheric carbon dioxide (BORGES *et al*., 2007, BANERJEE *et al*., 2019) and they are used as feedstock for biofuel production (DOAN *et al*., 2011, JAGADEVAN *et al*., 2018), consequently their large-scale cultivation could contribute to the establishment of a "Clean Development Mechanism", besides the production of biodiesel and other bioproducts of commercial interest (TANG *et al*., 2020).

2 MATERIAL AND METHODS

The experiments were performed with the marine microalgae *Nannochloropsis oculata* (clone NANN OCUL-1) and *Conticribra weissflogii* (clone THAL WEIS-1) inoculum obtained from the Laboratory of Phytoplankton and Aquatic Microorganisms of the Federal University of Rio Grande (FURG), southern Brazil.

The experiments were carried out in culture medium using salt water (salinity 34) and the fertilizers urea, ammonium sulphate and triple superphosphate (see tab. 1). For the experiment with *C. weissflogii* it was added 1mL L^{-1} of silicate solution ($Na_2SiO_3.5H_2O$ - 40g L^{-1}) to the medium.

Table 1. Composition of media for culture of *Nannochloropsis oculata* and *Conticribra weissflogii*.

Substance	Concentration
Salt water	1,000 ml
Ammonium sulphate	150 mg
Urea	7.5 mg
Triple superphosphate	25 mg

Fonte: Yamashita; Magalhães (1981)

Samples were distributed in the following treatments: 1) Control - culture medium with two nitrogen sources (urea and ammonium sulfate), 2) Ammonium - culture medium with only ammonium sulfate as nitrogen source, 3) Urea - culture medium only with urea as nitrogen source.

The tests were conducted in 500 ml Beckers, with three repetitions for each treatment. Cultures were maintained at 21°C, and with 3,500 lux of light and photoperiod of 12 L:12D. The experiment was finished when the species reached the senescent growth phase.

Every two days, samples (20 ml) were collected to determine the concentrations of total ammonia nitrogen ($N^{-}NH^{3}$ + $N^{-}NH^{4+}$) [14] (IOC, 1983) and urea (GOYENS et al. 1998).

Water samples for cells counting were fixed with Lugol's solution (Sournia 1978). The determination of microalgae abundance was conducted using a Neubauer chamber, under light microscope every two days.

Cell density and ammonium total data were compared statistically by one-way analysis of variance (ANOVA) ($P<0.05$). To compare the mean averages of cell density of treatments and control, we used the Student t test for independent samples (WILKINSON, 1998).

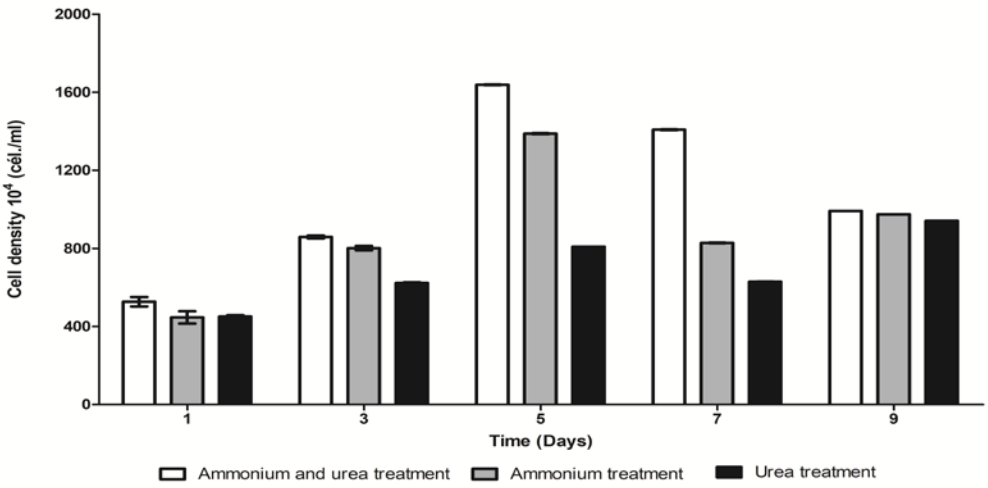
To evaluate the production costs of fertilizer solution we used market values of these major nutrients in February 2021.

3 RESULTS AND DISCUSSION

3.1 *Nannochloropsis oculata*

The highest cell abundance of *Nannochloropsis oculata* occurred in the control (1,638 ±20.20 x 10^4 cells. mL^{-1}) and in the ammonium (1,388 ±325.78 x 10^4 cells. mL^{-1}) treatments, on the fifth day. The urea treatment showed the lowest densities ranging from 451± 214.26 x 10^4 cells. mL^{-1} to 941 ± 41.63 x 10^4 cells. mL^{-1} (Fig. 1). There were no significant differences in cell density between treatments with ammonium sulfate and complete medium (Control) for *Nannochloropsis oculata* ($p=0.66$). Conversely, on the fifth day cell abundance in treatment with urea was significantly lower compared to complete medium (control) ($p=0.002$).

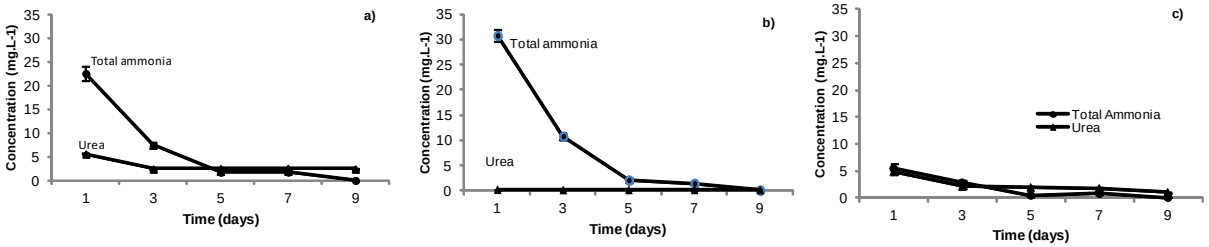
Figure 1. Variation in cell density (CEL. / mL) of *Nannochloropsis oculata*.



Fonte: Elaborated by the authors

Concentration of total ammonia decreased with the time in all treatments, ranging from 0.10 (± 0.03) to 24.30 (±0.20) mg . L^{-1} in the control treatment, 0.11 (± 0.02) to 30.77 (± 0.30) mg . L^{-1} in the treatment with ammonium sulfate and 0.11 (± 0.01) to 5.43 (± 0.03) mg . L^{-1} for urea treatment (Fig. 2).

Figure 2. Variation of total ammonia and urea concentration for *Nannochloropsis oculata* in the fertilizer medium. a) Complete medium with ammonium sulfate and urea. b) Medium with ammonium sulfate. c) Medium with urea



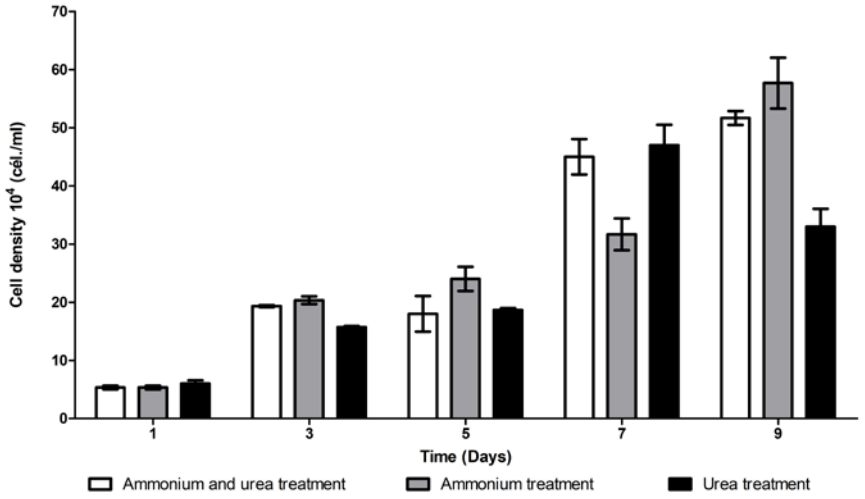
Fonte: Elaborated by the authors

The urea decreased over the experimental period, ranging from 2.49 (± 0.08) to 5.64 (± 0.170) mg . L^{-1} in the control treatment, 1.08 (± 0.104) to 4.85 (± 0.364) mg . L^{-1} in the urea treatment and between 0.19 (± 0.027) and 0.25 (±0.030) mg . L^{-1} in the ammonium treatment (Fig. 2).

3.2 *Conticribra weissflogii*

The highest cell density of *Conticribra weissflogii* was found on the 7th day in the ammonium treatment (57 ± 9 x 10^4 cells . mL^{-1}), followed by the control (50 ± 8.5 x 10^4 cells . mL^{-1}), and urea (42 ± 10.5 x 10^4 cells . mL^{-1}) treatments (Fig. 3). The results showed no significant difference in cell abundance between treatments with ammonium sulphate and complete medium (Control) (p=0.19).

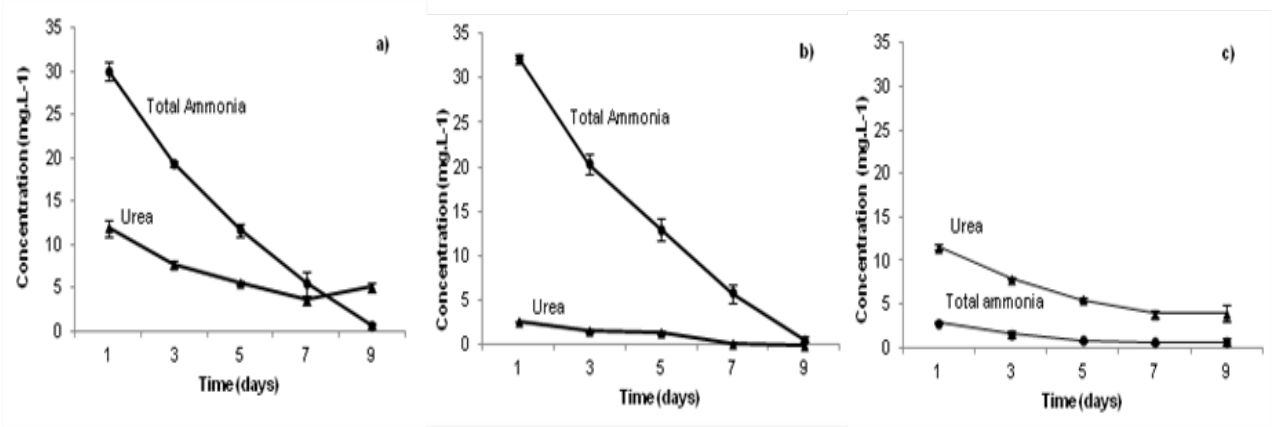
Figure 3. Variation in cell density (CEL. / mL) of *Conticribra weissflogii*



Fonte: Elaborated by the authors

The concentration of total ammonia gradually decreased along the time, with higher values for to control and ammonium treatments, ranging from 0.60 (± 0.37) to 30 (± 1.00) mg . L^{-1}(control) and 0.6 (± 0.34) to 32.17 (± 0.57) mg . L^{-1} (ammonium), while in the urea treatment this nutrient varied from 0.6 (± 0.10) to 2.90 (± 0.1) mg . L^{-1}(Fig. 4).

Figure 4. Variation in the concentration of total ammonia and urea for *Conticribra weissflogii* in the medium fertilizer. a) Complete medium with ammonium sulfate and urea. b) Medium with ammonium sulfate. c) Medium with urea.



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The urea concentration varied from 11.9 (± 0.34) to 3.59 (± 0.45) mg . L^{-1} (seventh day), increasing to 5.1 (± 0.57) mg . L^{-1} on the ninth day in the control treatment. In the other treatments the urea concentration decreased gradually over time, the medium with ammonium ranged from 2.6 (± 0.07) to 0.01 (± 0.03) mg . L^{-1} and in the medium with urea ranged from 11.5 (± 0.33) to 3.9 (± 0.9) mg . L^{-1} (Fig. 4).

The use of unsuitable culture medium may affect the growth rate and chemical composition of microalgae, altering the amount of protein, carbohydrates, lipids and pigments contents produced by the algae

(PAUW *et al.* 1984; BEGUM et al. 2007; LOURENÇO *et al.* 2002). Even simple of culture medium present various chemical elements and vitamins, but they always have large amounts of nitrogen and phosphorus (usually orthophosphate) in their composition (LAM; LEE, 2012). For large-scale microalgae production it is important diminish the costs of culture medium, although this must stimulate the growth of microalgae and achieve adequate chemical composition (PAUW *et al*., 1984). For some decades agricultural fertilizers have been tested as microalgae culture medium for large volumes production, with good results (YAMASHITA; MAGALHÃES, 1981; LOURENÇO, 2006; LAM; LEE, 2012).

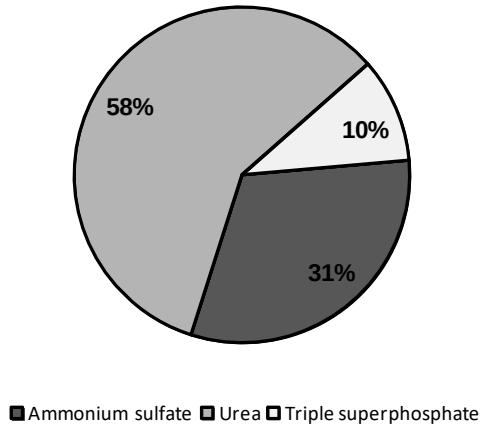
The most common nitrogen sources used in the large-scale microalgae cultivation are urea (organic) and ammonia (inorganic) that are marketed as fertilizers. However, the production of these nitrogen forms requires a large expenditure of energy, with high rates of emission of carbon dioxide (KIM; DALE 2005; GLIBERT *et al*. 2006). Therefore, it would be important to rationalize the use of nitrogen to the microalgae production, reducing the availability of nitrogen sources in the culture media to only one (ammonia or urea).

The results show clearly that there was no significant difference between the growth of *N.oculata* (clone NANN OCUL-1) and *C. weissflogii* (clone THAL WEIS-1) cultured with ammonium sulphate or urea media (Figs. 1, 3). This indicates that the changes in the sources of nutrients did not affect the growth rate of these clones during the 9th day of culture. Thus, urea could be eliminated from the culture medium without major consequences for the growth of both species.

This data agreed with others authors who suggest that agricultural fertilizers are excellent substitute that may be used for the production of large volumes of microalgae, due to the fact that there is no significant difference between the content of protein, carbohydrates and lipids and density cells of culture with both media (VALENZUELA-ESPINOZA, *et al.* 2002; NETO *et al*., 2018; COVELL *et al*., 2020,).

The fertilizer solution with two nitrogen sources (ammonia and urea) represent the highest cost in the medium production (R$ 3.79 or 90%). Therefore, the removal of urea would save ca 58% (R$ 2.47) of total costs (R$ 4.23) for production of 10,000 L of agricultural fertilizer media (Fig. 5).

Figure 5 Percentage costs of the compounds of agricultural fertilizer media.



Fonte: Elaborated by the authors

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Moreover, the elimination of urea from the massive microalgae culture media could reduce the cost for wastewater treatment that receive the effluents of microalgae production that is between R$ 0.68 and R$ 1.36 m^{-3} (MOHSENPOUR *et al*., 2021). Accelerate eutropphycation may lead to the formation of harmful algae blooms, with higher toxin production due to the presence of urea in the water (TRAINER *et al*., 2003; GLIBERT *et al*., 2005; THESSEN *et al*., 2009). The toxins may be accumulated through the food web and cause a serious disease (paralysis, amnesia, etc.) in higher-level consumers, including humans, birds and mammals (HOWARD, 2007).

4 CONCLUSIONS

This study demonstrates that the removal of urea of the medium agricultural fertilizers in microalgae culture has financial advantages, constitutes a viable alternative to reduce costs and this biotechnology is a feasible alternative method in the commercial aquaculture of microalgae.

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