PRODUCTION OF DOCOSAHEXAENOIC ACID (DHA) FROM *Thraustochytrium sp*. ATCC 26185 USING DIFFERENTS NITROGEN CONCENTRATIONS

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In this work it was studied the polyunsaturated fatty acids (PUFAs) production, especially DHA, from *Thraustochytrium* sp. ATCC 26185, under different total nitrogen (TN) availability. Three different TN conditions were evaluated: two with initial concentrations of 2.4 g/L and 0.8 g/L, and the third in a fed-batch process with a rate of 0.009 g/L.h. For each experiment the biomass, glucose, TN and PUFAs were determined. The major composition of the PUFAs in *Thraustochytrium* sp. ATCC 26185 cell biomass were DPA ω6 (21-25 %) and DHA (69-73 %), regardless of the type and time of culture. The maximum cell concentration (30.2 g/L) was obtained using 2.4 g/L TN in 168 h of culture. With this same concentration of TN it was possible to produce the highest concentration of DHA (1.16 g/L) in 120 h of culture, demonstrating that the growth of *Thraustochytrium* sp. ATCC 26185 and yield of PUFAs are dependent on the source concentration of TN available for consumption of this oleaginous microorganism, as well as culture time.

KEY-WORDS: DOCOSAHEXAENOIC ACID; LIPIDS; NITROGEN SOURCE; POLYUNSATURATED FATTY ACIDS; THRAUSTOCHYTRIDS.

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1 INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are among the nutrients of greatest interest because of their beneficial health effects and for their wide application in food and pharmaceutical products (SIJTSMA and SWAAF, 2004). In the human organism they are important for the reproductive, and immune system, in maintenance of cell membranes and the generation of prostaglandins (substances regulating inflammatory processes and blood coagulation). Additionally, PUFAs of ω3 and ω6 family reduce levels of low-density lipoprotein (LDL) or bad cholesterol because they modify the composition of cell membranes and lipoproteins, besides inducing increased fecal excretion of cholesterol and bile (RUXTON *et al*., 2005).

Among the PUFAs, docosahexaenoic acid (DHA, C22:6 ω3) and docosapentaenoic acid (DPA, C22:5 ω6) have noted importance for human cells. Clinical and epidemiological studies have indicated the DHA as the major part of the phospholipid membrane in the central nervous system cells, and is also found in high concentrations in the retina (22 to 33 % of total fatty acids) (DAS and FAMS, 2003; SILVA, MIRANDA-JÚNIOR and SOARES, 2007). Therefore, this fatty acid is essential for brain and visual development. The ω6 (DPA) is important for human health, as it prevents the onset of various diseases, such as cardiovascular accidents (myocardial infarction, thrombosis, atherosclerosis), diabetes, inflammation and rheumatism (arthritis, osteoporosis, asthma) (NAUROTH *et al*., 2010).

The main commercial sources of PUFAs, especially of DHA are oils of marine fish. However, its widespread use is limited for seasonal fishing variations, sea pollution and the high cost to process, extract and purify the oil (JIANG *et al*., 2004). Alternatively, some heterotrophic microorganisms can produce PUFAs in controlled environment. It removes the dependency on climatic and seasonal variables, keeping the production during the all year and reducing the cost of production (SWAAF, SIJTSMA and PRONK, 2003). Among these microorganisms, the oleaginous group *Thraustochytrids* is highlighted, since they can accumulate over 50 % of their weight as lipids, from which more than 25 % can be DHA (RAGHUKUMAR, 2008).

The lipid accumulation in oleaginous microorganisms normally occurs when the medium has a carbon source excess and limited amount of nitrogen. Thus, when the microorganism multiplies, the nitrogen source is rapidly exhausted, but it continues assimilating the carbon source that is channeled directly to the synthesis of lipids (RATLEDGE and WYNN, 2002). However, culture conditions involving low concentration of nitrogen decrease the cell growth reducing lipids and DHA yield (SHENE *et al*., 2010).

This study aimed to investigate the effect of total nitrogen concentration and culture conditions of the microorganism *Thraustochytrium* sp. ATCC 26185 for the production of PUFAs, especially DHA.

2 MATERIAL AND METHODS

2.1 MICROORGANISM

The *Thraustochytrium* sp. ATCC 26185 strain used in this study was obtained from American Type Culture Collection (Manassas, VA, USA).

2.2 PREPARATION OF INOCULUM

Cells from the microorganism *Thraustochytrium* sp. ATCC 26185 stored at 4 °C in potato dextrose agar were transferred to 500 mL flasks containing 100 mL medium composed (g/L) of: yeast extract (1.0), peptone (1.0) and glucose (5.0) in seawater (1.5 % w/v). The glucose was sterilized

separately. Cells were incubated in an orbital shaker (Ika, KS 260B) at 30 °C, 150 rpm, without light, for 48 h (FURLAN *et al*., 2012).

2.3 CULTURE CONDITIONS

The cultivations were carried out in bench bioreactor (Sartorius Stedim Biotech, Biostat® Bplus equipped with pressure flow meters and gases and liquids controllers) into the culture vessel borosilicate glass, with a capacity of 5 L, presenting the medium to following composition (g/L): KH_2PO_4 (1.54), $MgSO_4.7H_2O$ (2.62), NaCl (0.71), glucose (30.0) and different total nitrogen concentrations (TN): 2.4 g/L (6.25 g/L $(NH_4)_2SO_4$ and 8.8 g/L yeast extract), 0.8 g/L (1.89 g/L $(NH_4)_2SO_4$ and 3.23 g/L yeast extract) as initial condition in a batch process, and 0.009 g/L every hour in fed-batch process (0.021 g/L.h $(NH_4)_2SO_4$ and 0.036 g/L.h yeast extract). All components were solubilized in 3.15 L of seawater (1.2 %, w/v).

The extracted yeast sterilization and glucose were performed individually at 121 °C for 15 min in an autoclave (Cetorclav, CV-EL-18 L). The bioreactor was sterilized by autoclaving (Ajc, Uniclave 77-127 L) for 60 min and the remaining medium components were sterilized by membrane filtration (0.22 µm, Millipore).

The dissolved components (sterilized) were added into the bioreactor with solutions of metal (mg/L): $MnCl_2.4H_2O$ (3.0), $ZnSO_4.7H_2O$ (3.0), $CoCl_2.6H_2O$ (0.04), $Na_2MoO_4.2H_2O$ (0.04), $CuSO_4.5H_2O$ (2.0), $NiSO_4.6H_2O$ (2.0), $FeSO_4.7H_2O$ (10.0) and solution of vitamins (mg/L): Thiamine (9.5) and Calcium pantothenate (3.2) previously sterilized by membrane filtration (0.22 µm, Millipore). Finally, 350 mL of inoculum (10 % v/v with respect to the total volume of culture medium) were added.

The experiments were conducted at 23 °C with agitation of 100 rpm and pH 6.0, adjusted with NaOH (4 N). In the first 96 h of culture the dissolved oxygen concentration in the medium was maintained at 5 % saturation, controlled by aeration (0-2.5 vvm), followed by injection of 0.25 vvm pure oxygen. After this period, injections of air and oxygen were discontinued.

2.4 DETERMINATION OF THE BIOMASS CONTENT

The cell concentration was determined according to Min *et al*. (2012) with modifications at intervals of 24 h, filtering an aliquot of the culture medium on filter paper with glass microfiber (GF/C: 1.2 µm, Whatman) previously weighed. The biomass in the microfiber layer was washed twice with distilled water and dried at 60 °C in an oven (Memmert) for 24 h. The biomass content was determined by difference between initial and final weight.

2.5 DETERMINATION OF GLUCOSE

Sugars were measured in the culture supernatant at 24 h intervals by spectrophotometric method proposed by Miller (1959) using Uv/Vis dual beam absorption spectrophotometer (Ati Unicam Helios, Alpha, UK).

2.6 DETERMINATION OF TOTAL NITROGEN

The quantification of the total nitrogen content (defined and complex sources) was performed at intervals of 24 h, in the supernatants of the cultures, according to the procedure by Furlan *et al*. (2012).

2.7 DETERMINATION OF FATTY ACIDS PROFILE

Samples of the culture collected at intervals of 24 hours, were centrifuged (Kubota, 6800) at 8742 g for 15 min at 4 °C, and the biomass washed with distilled water and centrifuged again. This process was repeated twice. The biomass was frozen at -20 °C and dry for 48 h in a lyophilizer

(Heto, Power Dry LL 3000).

Lyophilized cell biomass between 20 and 100 mg was weighed and added to 50 µL of internal standard solution C23:0 (50 mg/mL) in order to express the results in g of fatty acid/g of biomass lyophilized. The methyl esters of fatty acids were prepared by esterification by acid catalysis using the method of Lepage and Roy (1986) modified by Cohen, Vonshak and Richmond (1988), analyzed by gas chromatograph (Varian, CP 3800) equipped with autosampler, injector and flame ionization detector (FID), both at 250 °C. The separation occurred using a polyethylene glycol capillary column DB-WAX (Agilent, 30 m length, 0.25 mm internal diameter and 0.25 µm thick) heated at 180 °C (5 min) gradually increasing every 4 °C/min up to 220 ºC (holding for 25 min) and in increase gradually (20 °C/min) to 240 ºC (holding for 15 min). The methyl esters were identified in the sample by comparison with the retention times of chromatographic patterns Sigma-Aldrich Co. (St. Louis, MO, USA).

Results were subjected to analysis of variance (ANOVA) and significant differences were identified by comparing the average level of 5 % significance. Before performing ANOVA it was necessary to check if the values were normal (Kolmogorov-Smirnov test) and homoscedastic (Cochran test) (TRIOLA, 2008).

3 RESULTS AND DISCUSSION

3.1 KINETICS OF GROWTH, GLUCOSE CONSUMPTION AND TOTAL NITROGEN

Figures 1, 2 and 3 show the average concentrations of the contents of cell biomass, glucose and total nitrogen during the cultivations of *Thraustochytrium* sp. ATCC 26185.

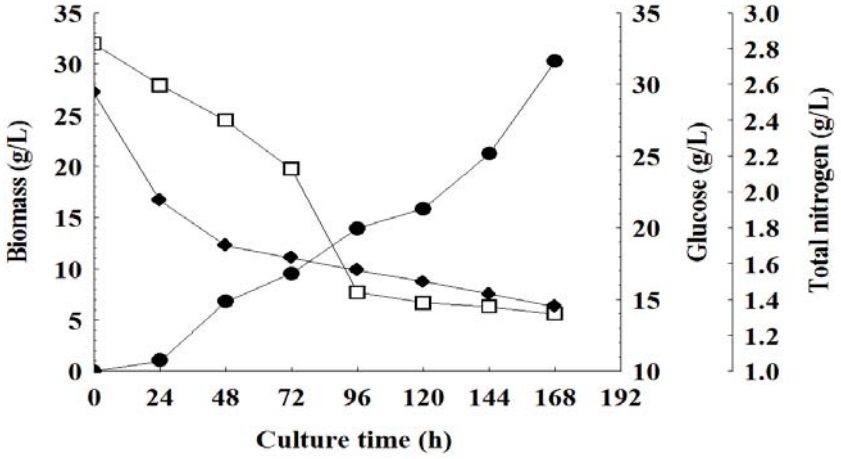

FIGURE 1 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE CULTIVATION OF *Thraustochytrium* sp. ATCC 26185, USING 2.4 g/L OF TOTAL NITROGEN

● Biomass (g/L); □ Glucose (g/L); ♦ Total nitrogen (g/L).

The experiment starting with total nitrogen concentration of 2.4 g/L (Figure 1) showed a maximum specific growth rate μ_{max} = 4.29/h, in the early stages of cultivation and a generation time (tg) of 0.16 h. Furthermore, it was observed that the maximum biomass concentration (30.2 g/L) was reached after 168 h of culture, with average productivity 0.18 g/L.h of cell biomass. The average glucose consumption for this initial condition was 0.11 g/L.h and the highest specific speed of consumption of this substrate 0.67/h after the first 24 h of culture. This experiment also demonstrated that, each gram of glucose produced 1.6 g of biomass ($Y_{Biomass/Glucose}$: 1.6). The nitrogen supply had maximum specific consumption rate of 1.34/h recorded at the baseline, with an average consumption

of 0.007 g/L.h. Featuring a conversion factor of biomass substrate in 25.3 ($Y_{Biomass/Nitrogen}$).

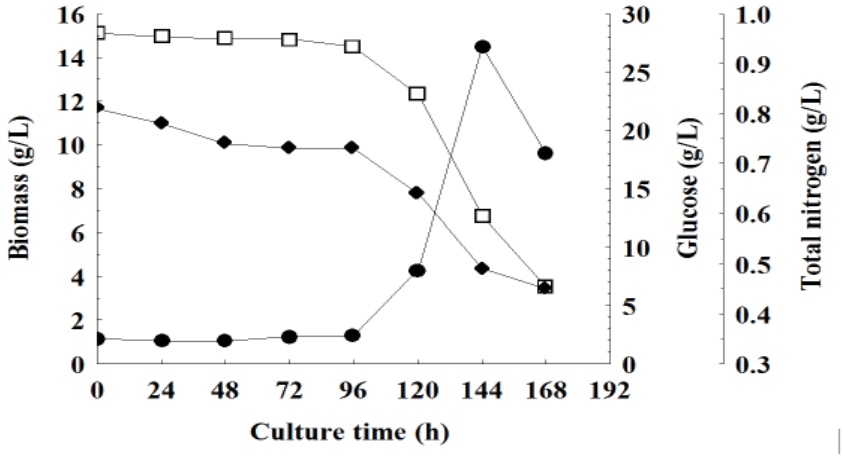

FIGURE 2 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE CULTIVATION OF *Thraustochytrium* sp. ATCC 26185, USING 0.8 g/L OF TOTAL NITROGEN

● Biomass (g/L); □ Glucose (g/L); ♦ Total nitrogen (g/L).

Results from the second experiment (Figure 2), with 0.8 g/L initial total nitrogen concentration, showed highest biomass content (14.5 g/L) after 144 h of culture, and average biomass productivity of 0.11 g/L.h. Under this initial conditions, the biomass had μ_{max} = 0.09/h at 106 h, and a cell doubling time of 7.4 h. The maximum specific rate of glucose consumption (0.07/h) was 101 hours, with an average consumption of 0.13 g/L.h. This culture showed conversion from glucose to biomass $Y_{Biomass/Glucose}$ = 0.88. The highest specific speed (0.09/h) of total nitrogen consumption was reached at 20 h, with average consumption of 0.002 g/L.h. Each gram of nitrogen consumed was converted into 46.4 g of biomass ($Y_{Biomass/Nitrogen}$).

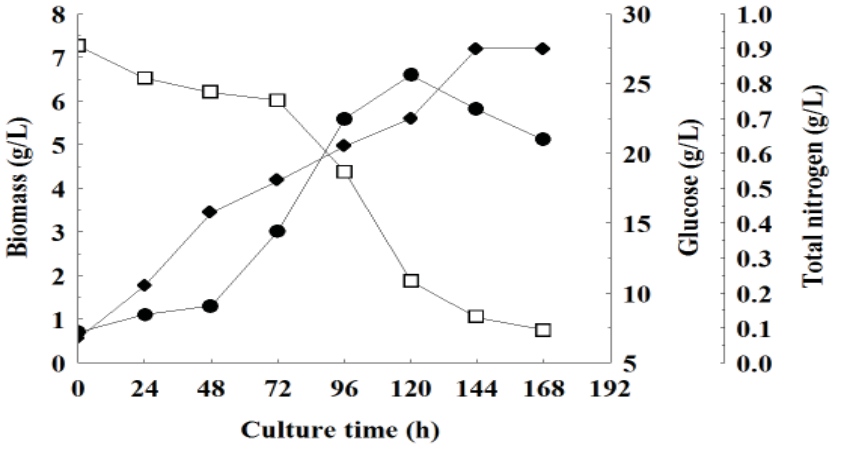

FIGURE 3 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE GROWING *Thraustochytrium* sp. ATCC 26185, EMPLOYING FED-BATCH SYSTEMS 0.009 g/L (HOURLY)OF TOTAL NITROGEN

● Biomass (g/L); □ Glucose (g/L); ♦ Total nitrogen (g/L).

The nitrogen supply by fed-batch process (0.009 g/L.h TN) showed the highest concentration of biomass (6.6 g/L) at 120 h (Figure 3), with a maximum biomass productivity of 0.05 g/L.h. The maximum specific cell growth 0.03/h was observed at 55 h of cultivation. Furthermore, in this

experiment the time required for cell replication was 24.8 h. At time 90 hours occurred the highest specific rate of glucose consumption (0.06/h) with an average consumption of 0.12 g/L.h, and conversion factor from glucose to biomass $Y_{biomass/Glucose}$ = 0.35. Maximum specific consumption of total nitrogen 0.02/h was at 6.2 h of cultivation, the average consumption of this substrate was 0.003 g/L.h. The conversion factor $Y_{biomass/Nitrogen}$ = 16.4, indicates that each gram of nitrogen produced 16.4 g of biomass.

Among tested TN nitrogen conditions, the initial concentration of 2.4 g/L had the greatest cell concentration (30.2 g/L), the highest specific speed of substrate consumption and the shortest time required for cell duplication (tg = 0.16 h). The highest consumption of total nitrogen (0.007 g/L.h) observed in this condition induced higher cell productivity (0.18 g/L.h) than the other experiments, since nitrogen promotes the synthesis of nucleic acids and proteins and consequently the cell division (BURJA *et al.*, 2006).

Ganuza, Anderson and Ratledge (2008) evaluated the use of ammonium tartrate as nitrogen source in the culture medium of *Schizochytrium* sp. and found that the microorganism grew to 23.8 g/L in 48 hours, providing 1.85 g/L TN. In the study by Chen *et al*. (2010) the maximum cell concentration (9.27 g/L) was obtained when cultured *Aurantiochytrium* sp. BR-MP4-A1 with 2.4 g/L of TN. Min *et al*. (2012) studied the effect of cultivation conditions on the growth of *Thraustochytrium roseum* ATCC 34303 and reached a maximum cellular biomass content of 7.9 g/L, employing glucose concentration of 30 g/L and 0.6 g/L total nitrogen (yeast extract and peptone).

At Figures 2 and 3 it is possible to see that this microorganism under studied conditions, reached maximum values of cell biomass in the times of 144 and 120 h, respectively, with subsequent decrease. This biomass decreasing can be related to the inhibition of this microorganism caused by the formation of acidic products during cultivation. McCormick (1995) reported that environmental conditions are becoming increasingly unsuitable for cells to survive over time. Another reason would be cells death caused by nutrients deficit. The low concentrations of glucose and TN, evidenced in these experiments, can be one of the reasons for cells decline, since their concentrations at 168 h of culture (Figures 2 and 3) were about half of the quantified amount in the experiment using 2.4 g/L TN at the same time (Figure 1).

In Figure 3, is also possible to see an accumulation of TN along the cultivation time, since the TN input (fed-batch) was greater than the amount required for the cell development and maintenance.

The fed-batch cultivation consumed approximately the same amounts of substrate (0.12 g/L.h of glucose and 0.003 g/L.h of nitrogen) as the cultivation with inital total nitrogen 0.8 g/L (0.13 g/L.h of glucose and 0.002 g/L.h of nitrogen). However, its conversion efficiency in cellular biomass, 0.05 g/L.h, was different from experiment with 0.8 g/L TN, 0.11 g/L.h. Possibly this occurred because part of the nutrients have been used only for cellular maintenance and not for favoring cell multiplication. It is noteworthy that the time required for cell replication tg = 24.8 h in the fed-batch experiment was superior to 7.4 h observed when using 0.8 g/L TN at the cultivation starts.

3.2 FATTY ACID PROFILE

Figure 4 shows the average values of the contents of PUFAs versus time of cultivation of *Thraustochytrium* sp. ATCC 26185.

After application of ANOVA and the test to compare means, Tukey at 5 % significance level, the results of PUFAs (g/L) production showed significant difference between the times of cultivation in the three nitrogen concentrations studied, except in times of 96 and 144 h for the cultivation which used 2.4 g/L of total nitrogen and from 24 to 96 h for the experiment that used 0.8 g/L of TN. The highest yield of PUFAs (1.68 g/L) was observed in the experiment providing initial TN of 2.4 g/L after 120 h of cultivation. The cultivation with 0.8 g/L TN had greatest amount of PUFAs of 0.94 g/L at 144 h; while under the TN fed-batch process it was 0.65 g/L at 96 h (Figure 4).

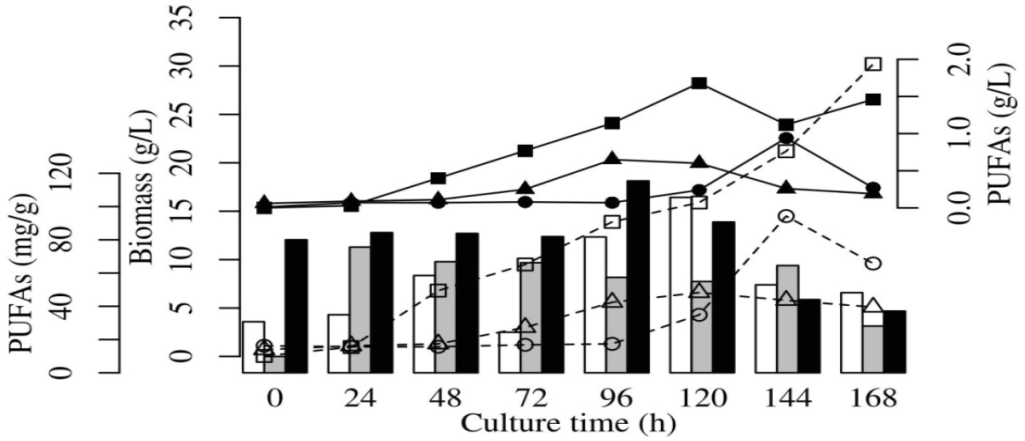

FIGURE 4 - PUFAs CONTENT IN THE BIOMASS OF *Thraustochytrium* sp. ATCC 26185. TN: TOTAL NITROGEN

PUFAs (mg/g): □ 2.4 (g/L) TN □ 0.8 (g/L) TN ■ 0.009 (g/L.h) TN.
PUFAs (g/L): -■- 2.4 (g/L) TN -●- 0.8 (g/L) TN -▲- 0.009 (g/L.h) TN.
BIOMASS (g/L): ⊟ 2.4 (g/L) TN ⊖ 0.8 (g/L) TN △ 0.009 (g/L.h) TN.

The Figure 5 shows the fatty acid profile for each experiment in periods where the highest yields were achieved in PUFAs (g/L).

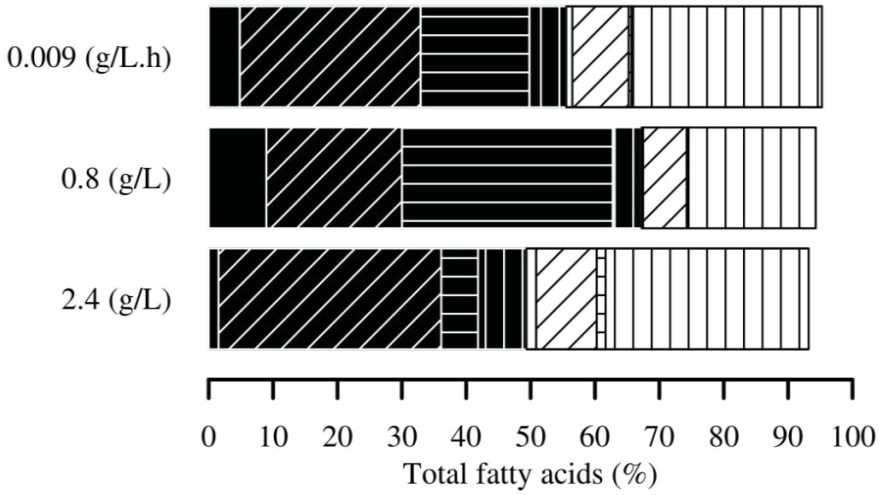

FIGURE 5 - COMPOSITION OF FATTY ACIDS OF CELL BIOMASS OF *Thraustochytrium* sp. ATCC 26185 DURING 120 h (2.4 g/L TOTAL NITROGEN), 144 h (0.8 g/L TOTAL NITROGEN) AND 96 h (0.009 g/L.h TOTAL NITROGEN)

■ C14:0 ▨ C15:0 ▬ C16:0 ▮ C17:0 □ C20:5 ω3 ▨ C22:5 ω6 (DPA) ▤ C22:5 ω3 ▥ C22:6 ω3 (DHA).
Dark bar: percentage of saturated fatty acids.
Light bar: percentage of PUFAs.

Evaluating the experiment with initial TN concentration of 2.4 g/L at 120 h of culture, it was observed that 10.5 % (w/w) of cell biomass were PUFAs, with 21 % of these being DPA ω6 (Figure 5) that is 2.2 % of the total biomass (0.35 g/L). It can also be seen that 69 % of PUFAs were DHA

(Figure 5), that is 7.3 % of biomass (1.16 g/L). At 144 hours, in the cultivation that employed 0.8 g/L of initial TN, 6.5 % (w/w) of cell biomass was composed of PUFAs, and 25 % was DPA ω6 (Figure 5) that is 1.6 % biomass content (0.23 g/L). Also it was observed that 73 % of DHA was PUFAs (Figure 5) or 4.7 % of the total biomass (0.68 g/L). While in the fed-batch culture, 0.009 g/L.h TN at 96 hours, 11.6 % (w/w) of the biomass consisted of PUFAs, 21 % of these were DPA ω6 (Figure 5) or 2.5 % of the total biomass (0.14 g/L). It can also be seen that 73 % of DHA was PUFAs (Figure 5) or 8.4 % of the total biomass (0.47 g/L).

In this study, among the fatty acids a high contents of C15:0 (21-35 %) and C16:0 (5-33 %), which are saturated, were found (Figure 5). The presence of fatty acid C15:0 in lipids produced by strains of *Thraustochytrids* has also been reported by other authors (KAMLANGDEE and FAN, 2003; CHANG *et al*., 2011). These results show the importance of propionate in the metabolic pathway of this microorganism (VLAEMINCK *et al*., 2006).

DHA was the predominant PUFA, ranging from 20 to 31.5 % of total fatty acids (Figure 5). Similar results were observed in the study by Scott *et al*. (2011), where the content of C16:0 was 33 % and DHA 36 %, relative to the total fatty acids, using *Thraustochytrium* sp. ONC-T18 after 5 days of cultivation. These authors also observed that DHA and DPA ω6 were the main PUFAs detected, as in the present study (Figure 5).

Observing the major fatty acids which form the PUFAs biomass, irrespective of the type and culture time, distribution of DPA ω6 (21-25 %) was approximated as well as that of DHA (69-73 %) with little variation (Figure 5).

The main comercial sources of PUFAs are species of fatty fish, such as herring, mackerel, salmon and sardines. Morais (2000), examined the lipid profile of sardine oil, and found that 31.1 % of fatty acids present were PUFAs with DHA content correspondent to 11 % of total fatty acids.

The strain used in this study showed higher concentrations of PUFAs (27-44 %) than the sardine oil (31.1 %) evaluate by Morais (2000), and the DHA concentration (20-31.5 %) produced by oleaginous microorganism was 2 to 3 times higher than the one found in the sardines oil (11%).

Cultivation of *Thraustochytrium* sp. 26185 to obtain oils rich in PUFAs is a promising alternative, because the use of fish on a large-scale is limited due to seasonal variations and the composition of the lipid content and fatty acids in fish varies according to species, sex, size, reproductive cycle, season, catch location, diet and nutritional status (GONÇALVES, 2011). In addition, the fish oil presents a great diversity of fatty acids with different chain lengths and degree of unsaturation, requiring an expensive process of extraction and purification (JIANG *et al*., 2004).

The fed-batch process experiment accumulated greater amounts of PUFAs (11.6 %) in relation to cell weight than other cultivations. This accumulation can be due to the low content of nitrogen supply over time. According to Shene *et al*. (2010), a limited amount of substrate favors the accumulation of lipids by oleaginous microorganisms. Ganuza and Izquierdo (2007) observed that the greatest accumulation of fatty acids in the biomass of *Schizochytrium* sp. G13/2S were between 28-30 % (w/w), employing low concentrations of monosodium glutamate (2-4 g/L) as nitrogen source and when this concentration was increased to 6 g/L, production of fatty acids decreased, because the cells accumulated nitrogen compounds. However, cultivation in fed-batch process had lower DHA (0.47 g/L) and DPA ω6 (0.14 g/L) yields, due to lower cell concentration (5.6 g/L) obtained when compared to that of cultivations which used 2.4 g/L TN (15.9 g/L biomass) and 0.8 g/L TN (14.5 g/L of biomass). The production of fatty acids such as DPA ω6 and DHA is dependent on accumulation of PUFAs, as well as the accumulation of lipid in the biomass. Thus, the yield of fatty acids is also related to the cellular concentration of microorganism at a given time. According to Shene *et al*. (2010) cultivation conditions involving low concentrations of TN, decrease cell growth, thus lower yields of lipid and DHA are obtained. This may be a cause of decrease of the content of PUFAs in final stage of cultivation of the strain of *Thraustochytrium* sp. ATCC 26185 seen in Figure 4.

Burja *et al*. (2006) evaluated different concentrations of nitrogen in the culture medium of *Thraustochytrium* sp. ONC-T18, and found that at the highest concentration of TN studied (1.24 g/L) 1.56 g/L of DHA was obtained, 6.7 % (w/w) of cell biomass.

4 CONCLUSION

The highest content of cell biomass (30.2 g/L) was obtained using 2.4 g/L of total nitrogen at 168 h of cultivation.

The majority of PUFAs found in biomass of *Thraustochytrium* sp. ATCC 26185 were DPA ω6 (21-25 %) and DHA (69-73 %), whose percentages did not show large variations with different growth conditions studied.

The highest yield of PUFAs (1.68 g/L) obtained from *Thraustochytrium* sp. ATCC 26185 was after 120 h of cultivation, giving 2.4 g/L of total nitrogen in a batch process. Under the same conditions, the highest concentrations of DHA (1.16 g/L) and DPA ω6 (0.35 g/L) were recorded.

This study showed that the growth of *Thraustochytrium* sp. ATCC 26185 and production of PUFAs, especially DHA are dependent on the concentration of the nitrogen source available for the consumption of this oleaginous microorganism, as well as cultivation time. Therefore, parameters such as medium composition and environmental factors should be considered to increase production of PUFAs.

RESUMO

PRODUÇÃO DE ÁCIDO DOCOSAHEXAENOICO (DHA) POR *Thraustochytrium* sp. ATCC 26185 UTILIZANDO DIFERENTES CONCENTRAÇÕES DE NITROGÊNIO

O objetivo deste trabalho foi estudar a produção de ácidos graxos poliinsaturados (PUFA), especialmente DHA, a partir de *Thraustochytrium* sp. ATCC 26185, sob diferentes condições de disponibilidade de nitrogênio total (NT). Três diferentes condições de NT foram avaliadas: duas com concentração inicial de 2,4 g/L e 0,8 g/L e a terceira em processo de batelada alimentada com taxa de 0,009 g/L.h. Para cada experimento foram determinados os conteúdos de biomassa celular, consumo de glicose, NT e PUFA. A composição majoritária dos PUFA na biomassa celular de *Thraustochytrium* sp. ATCC 26185 constituiu-se de DPA ω6 (21-25 %) e DHA (69-73 %), independente do tipo e tempo de cultivo. Obteve-se concentração celular máxima (30,2 g/L) utilizando 2,4 g/L de NT em 168 h de cultivo. A mesma concentração de NT produziu a maior concentração de DHA (1,16 g/L) em 120 h de cultivo, demonstrando que o crescimento de *Thraustochytrium* sp. ATCC 26185 e o rendimento em PUFA são dependentes da concentração da fonte de NT disponível para o consumo desse micro-organismo oleaginoso, assim como do tempo de cultivo.

PALAVRAS-CHAVE: ÁCIDO DOCOSAHEXAENOICO; LIPÍDIOS; FONTE DE NITROGÊNIO; ÁCIDOS GRAXOS POLIINSATURADOS; THRAUSTOCHYTRIDS.

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