PRODUCTION OF DOCOSAHEXAENOIC ACID (DHA) FROM THRAUSTOCHYTRIUM SP. ATCC 26185 USING DIFFERENTS NITROGEN CONCENTRATIONS

VALCENIR JÚNIOR MENDES FURLAN*
MARIA DO CASTELO PAULO**
VICTOR WEGNER MAUS***
JÚLIA FERREIRA****
IRINEU BATISTA*****
NARCISA MARIA BANDARRA******

Polyunsaturated fatty acids (PUFAs) of the type ω3 and ω6 play important physiological functions in human organism, since they are components of cell membranes and brain cells; they decrease the levels of triglycerides and can prevent the incidence of coronary heart disease. Various parameters, including concentration of the nitrogen source in the cultivation of oleaginous microorganisms have been reported to be essential in the biosynthesis and accumulation of PUFAs. The objective of this work is to study the effect of different concentrations of total nitrogen (TN) in the production of PUFAs, especially DHA, from *Thraustochytrium* sp. ATCC 26185. The concentrations of TN evaluated were 2.4 and 0.8 g/L (batch) and 0.009 g/L (hourly) under fed-batch process. The content of cell biomass, glucose consumption, TN and production of PUFAs was 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 obtained (30.2 g/L) was 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 concentration TN source available for consumption of this oleaginous microorganism, as well as culture time.

KEYWORDS: DOCOSAHEXAENOIC ACID; LIPIDS; NITROGEN SOURCE; POLYUNSATURATED FATTY ACIDS; THRAUSTOCHYTRIDS.

* Engenheiro de Alimentos, Doutor em Engenharia e Ciências de Alimentos, Docente da Universidade Federal do Pampa (UNIPAMPA), Itaqui, RS, Brasil (e-mail: juniorfurlan@unipampa.edu.br).

** Engenheira Biotecnológica, Pesquisadora da Empresa Depsiextracta Biological Technologies-Lda, Taipadas-Canha, Portugal (e-mail: mariacastelo@depsiextracta.com).

*** Engenheiro Ambiental, Doutorando em Ciência do Sistema Terrestre no Instituto Nacional de Pesquisas Espaciais (INPE), Brasil (e-mail: vwmaus1@gmail.com).

**** Técnica em Química, Pesquisadora do National Institute of Biological Resources (INRB/IPMA), Lisboa, Portugal (e-mail: jferreira@ipma.pt).

***** Engenheiro Químico, Mestre em Química Orgânica Tecnológica, Pesquisador do National Institute of Biological Resources (INRB/IPMA), Lisboa, Portugal (e-mail: irineu@ipma.pt).

****** Química, Doutora em Biotecnologia, Pesquisadora do National Institute of Biological Resources (INRB/IPMA), Lisboa, Portugal (e-mail: narcisa@ipma.pt).

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 LDL (low-density lipoprotein 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) may be noted.

Clinical and epidemiological studies have indicated that the DHA is a major component of the phospholipid membrane of the cells of the central nervous system, and is also found in high concentrations in the retina (22 to 33 % of total fatty acids) (DAS and FAMS, 2003; SILVA *et al*., 2007). Therefore, this fatty acid is critical for brain and visual development of newborns.

Like DHA, 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 these compounds, especially of DHA are oils of marine fish. However, its widespread use is limited due to seasonal variations of fish, marine pollution and the high cost of the process of extraction and purification of this oil (JIANG *et al*., 2004).

As alternative, heterotrophic microorganisms arise, they do not depend on climatic variation, and can be controlled, resulting in high cellular concentrations, reducing the cost of production (SWAAF *et al*., 2003). Among these microorganisms, the group *Thraustochytrids* is highlighted, considered oleaginous, since they can accumulate over 50 % of their weight in the form of lipids, of which more than 25 % can be DHA (RAGHUKUMAR, 2008).

The lipid accumulation in oleaginous microorganisms normally occurs when the medium contains excess carbon source and a 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 cell growth thus lower yields are obtained in lipids and DHA (SHENE *et al*., 2010).

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

2 MATERIALS 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 ºC 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 ºC , 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 concentrations of total nitrogen (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) 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 sterilization of yeast extract and glucose were performed individually at 121 ºC 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, was added 350 mL of inoculum (10 % v/v with respect to the total volume of culture medium).

The experiments were conducted at 23 ºC 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 ºC in an oven (Memmert) for 24 h. The biomass content was determined by difference between the 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 ºC , and the biomass washed with distilled water and centrifuged again.

This process was repeated twice. The biomass was frozen at -20 ºC 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 *et al*. (1988), analyzed by gas chromatograph (Varian, CP 3800) equipped with autosampler, injector and flame ionization detector (FID), both at 250 ºC . 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 ºC (5 min) gradually increasing every 4 ºC /min up to 220 ºC (holding for 25 min) and in increase gradually (20 ºC /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) and their variances were equal to (Cochran) (TRIOLA, 2008).

3 RESULTS AND DISCUSSION

3.1 KINETICS OF GROWTH, GLUCOSE CONSUMPTION AND TOTAL NITROGEN

Figure 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.

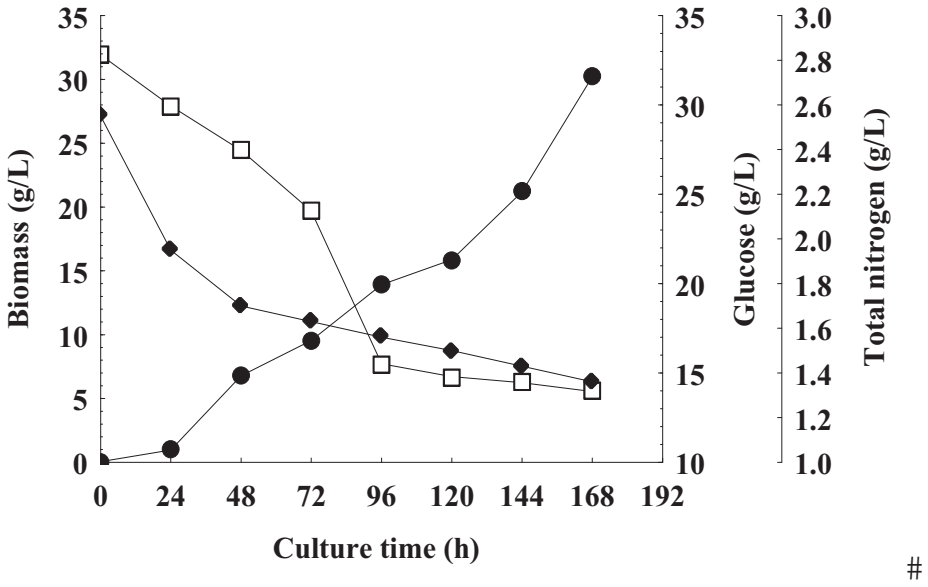

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FIGURE 1 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE CULTIVATION OF *THRAUSTOCHYTRIUM* SP. ATCC 26185, USING 2.4 G/L OF TOTAL NITROGEN

The experiment which provided 2.4 g/L total nitrogen (Figure 1) showed a maximum specific (μ_{max}) cell growth of 4.29/h, in the early stages of cultivation and a generation time (tg) of 0.16 h. Furthermore, one can observe that the maximum biomass concentration (30.2 g/L) was reached after 168 h of culture, with a productivity of 0.18 g/L.h of cell biomass.

The average consumption of glucose in this assay was 0.11 g/L.h and highest specific speed of consumption of this substrate (0.67/h) was after the first 24 h of culture. This experiment also demonstrated that, for every gram of glucose consumed, 1.6 g of biomass were produced ($Y_{Biomass/Glucose}$: 1.6).

For the supplied nitrogen, the maximum specific consumption rate was 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 ($Y_{Biomass/Nitrogen}$).

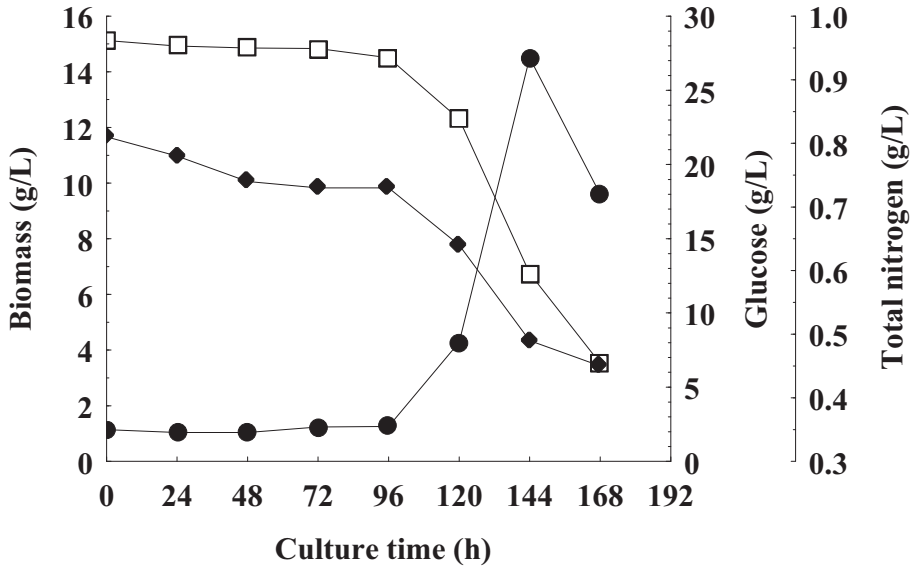

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FIGURE 2 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE CULTIVATION OF *THRAUSTOCHYTRIUM* SP. ATCC 26185, USING 0.8 G/L OF TOTAL NITROGEN

Biomass (g/L) Glucose (g/L) Total nitrogen (g/L)

From Figure 2 it can be seen that the highest content of biomass (14.5 g/L) was reached after 144 h of culture, employing 0.8 g/L of total nitrogen, with an average productivity 0.11 g/L.h biomass. At this concentration of TN used, the μ_{max} (0.09/h) of cell growth was recorded at 106 h, under these same conditions there was 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 a conversion of glucose to biomass ($Y_{Biomass/Glucose}$) of 0.88.

At 20 h the highest specific speed (0.09/h) of total nitrogen consumption was reached, with average fuel consumption with this substrate of 0.002 g/L.h. Each gram of nitrogen consumed was converted into 46.4 g of biomass ($Y_{Biomass/Nitrogen}$).

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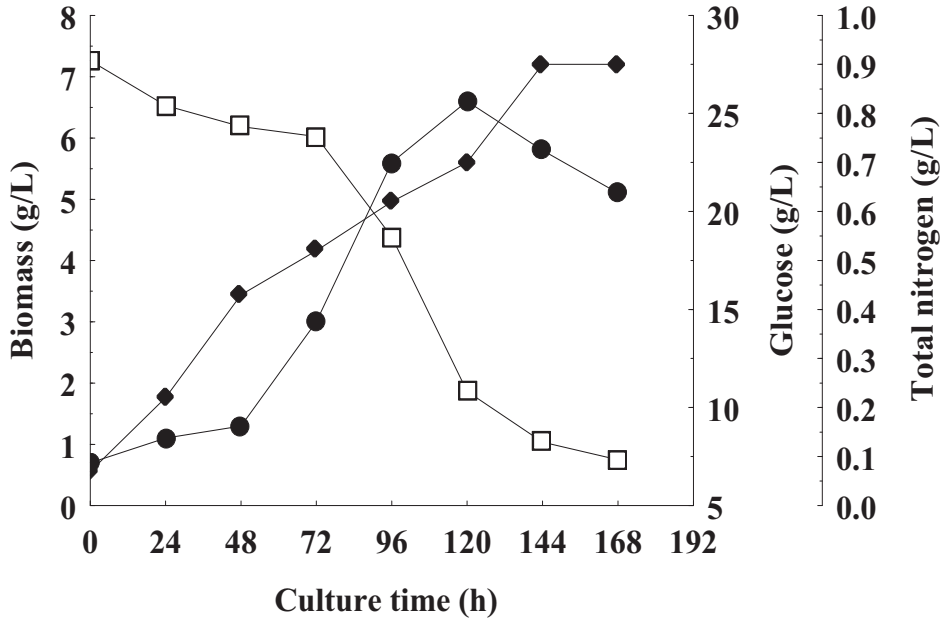

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FIGURE 3 - CONCENTRATIONS OF BIOMASS, GLUCOSE AND TOTAL NITROGEN DURING THE GROWING *THRAUSTOCHYTRIUM* SP. ATCC 26185, EMPLOYING FED-BATCH SYSTEMS 0.009 G/L (HOURLY) OF TOTAL NITROGEN

Biomass (g/L) Glucose (g/L) Total nitrogen (g/L)

Supplying nitrogen under fed-batch process (0.009 g/L.h TN), it was found that the highest concentration of biomass (6.6 g/L) was obtained at 120 h (Figure 3), with a maximum productivity of 0.05 g/L.h of cell biomass. At 55 h of cultivation maximum specific cell growth (0.03/h) was observed. Furthermore, the time required for cell replication to occur in this experiment was 24.8 h.

At time 90 hours, the highest specific rate of glucose consumption occurred (0.06/h) with an average consumption of 0.12 g/L.h and conversion factor (0.35) glucose into biomass ($Y_{Biomass/Glucose}$).

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), indicated that for each gram of nitrogen consumed 16.4 g of biomass was produced.

The experiment which provided 2.4 g/L of total nitrogen to the culture medium, obtained the maximum cell concentration (30.2 g/L), as the strain of *Thraustochytrium* sp. 26185 registered in the cultivation speeds specific substrate consumption and the shortest time required for cell duplication (tg: 0.16 h). The higher consumption of total nitrogen (0.007 g/L.h) observed in this condition gave better cell productivity (0.18 g/L.h) when compared to other experiments, since nitrogen promotes the synthesis of nucleic acids and proteins and consequently the cell division (BURJA *et al*., 2006).

Ganuza *et al*. (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).

From Figure 2 and 3 it is noted that this microorganism under the conditions studied, reached maximum values of cell biomass in the times of 144 and 120 h of culture, respectively, with subsequent decrease. This decrease in biomass content can be related to the inhibition of this microorganism caused by the formation of acidic products during cultivation, since McCormick (1995) reported that environmental conditions are becoming increasingly unsuitable for the cells to survive over time. Another reason would be the cell death caused by the deficiency of a nutrient medium. Low concentrations of glucose and TN evidenced in these experiments can be one of the reasons for this cell decline, since these values at 168 h of culture (Figure 2 and 3) were approximately half of those that were quantified in the experiment which used 2.4 g/L TN at the same time (Figure 1).

In Figure 3, one can also see an accumulation of TN along the cultivation, since the amount delivered (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 nitrogen) used as the cultivation that used 0.8 g/L of total nitrogen (0.13 g/L.h glucose and 0.002 g/L.h nitrogen). However, its conversion efficiency in cellular biomass (0.05 g/L.h) was not observed as in the experiment with 0.8 g/L TN (0.11 g/L.h). Possibly this occurred because part of 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 experiment under fed-batch process was superior to 7.4 h (tg) which used the cultivation 0.8 g/L TN.

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.

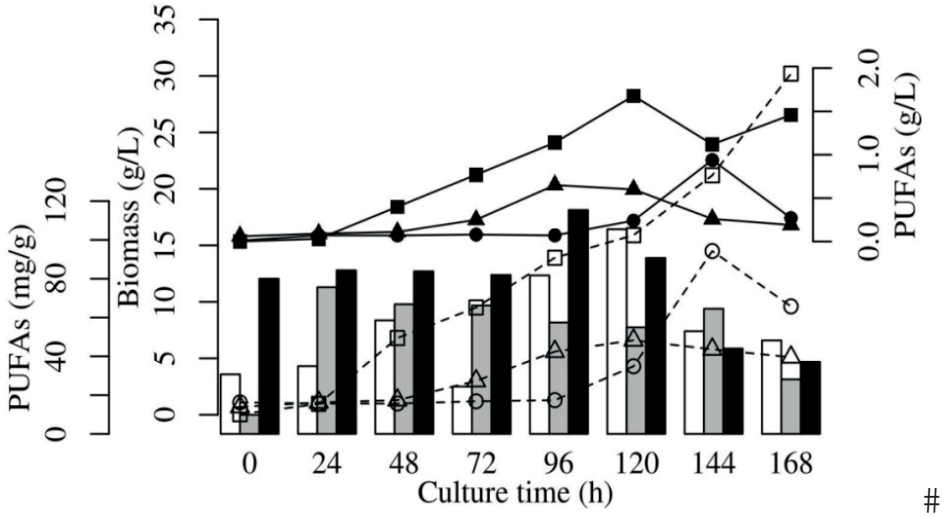

FIGURE 4 - PUFAS CONTENT IN THE BIOMASS OF *THRAUSTOCHYTRIUM* SP. ATCC 26185. TN: TOTAL NITROGEN

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

From the results of PUFAs (g/L) it was possible to apply ANOVA, followed by the comparison test between means (Tukey) at 5 % significance level, which we can conclude that there was 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 that provided 2.4 g/L of total nitrogen after 120 h of cultivation. In the cultivation with 0.8 g/L TN, the greatest amount of PUFAs (0.94 g/L) was obtained at 144 h; while under cultivation in the fed-batch process was at 96 h that the maximum production of PUFAs (0.65 g/L) was recorded (Figure 4).

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

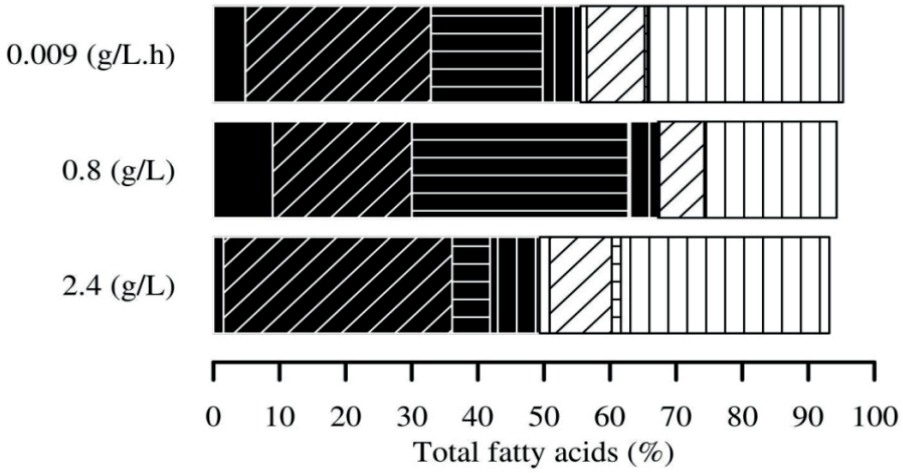

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FIGURE 5 - COMPOSITION OF FATTY ACIDS OF CELL BIOMASS OF *THRAUSTOCHYTRIUM* SP. ATCC 26185 DURING 120 H (2.4 G/L TOTAL NITROGEN), 144 H (0.8 G/L TOTAL NITROGEN) AND 96 H (0.009 G/L.H TOTAL NITROGEN)

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

In the experiment which utilized 2.4 g/L of nitrogen (120 h of culture), 10.5 % (w/w) of cell biomass consisted of 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).

In the cultivation that employed 0.8 g/L of TN at point 144 h, 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). One can also observe that 73 % of DHA was PUFAs (Figure 5) or 4.7 % of the total biomass (0.68 g/L).

While in culture which provided 0.009 g/L.h nitrogen (96 h point), 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 content of C15:0 (21-35 %) and C16:0 (5-33 %) which are saturated was found (Figure 5). The presence of fatty acid C15:0 in lipids produced by strains of *Thraustochytrids*, has 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 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 supplied 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 CONCLUSIONS

Using 2.4 g/L of total nitrogen reached the highest content of cell biomass (30.2 g/L) 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.

PRODUÇÃO DE ÁCIDO DOCOSAHEXAENÓICO (DHA) POR *THRAUSTOCHYTRIUM* SP. ATCC 26185 UTILIZANDO DIFERENTES CONCENTRAÇÕES DE NITROGÊNIO

RESUMO

Os ácidos graxos poliinsaturados (PUFAs) dos tipos ω3 e ω6 desempenham funções fisiológicas importantes ao organismo humano, uma vez que são componentes das membranas celulares e de células cerebrais, diminuem os níveis de triglicerídios e podem prevenir as incidências de doenças coronarianas. Diversos parâmetros, incluindo a concentração da fonte de nitrogênio no cultivo de micro-organismos oleaginosos, têm sido relatados por serem fundamentais na biossíntese e no acúmulo de PUFAs. Este trabalho teve como objetivo, estudar o efeito de diferentes concentrações de nitrogênio total (NT) para a produção de PUFAs, especialmente DHA, a partir de *Thraustochytrium* sp. ATCC 26185. As concentrações de NT avaliadas foram: 2,4 e 0,8 g/L (processo descontínuo) e 0,009 g/L (a cada hora) sob processo descontínuo alimentado. Foram determinados os conteúdos de biomassa celular, consumo de glicose, NT e a produção de PUFAs. A composição majoritária dos PUFAs na biomassa celular de *Thraustochytrium* sp. ATCC 26185 foram DPA ω6 (21-25 %) e DHA (69-73 %), independente do tipo e tempo de cultivo. A concentração celular máxima obtida (30,2 g/L) foi utilizando 2,4 g/L de NT em 168 h de cultivo, com esta mesma concentração de NT foi possível produzir 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 PUFAs são dependentes da concentração da fonte de NT disponível para o consumo deste micro-organismo oleaginoso, assim como do tempo de cultivo.

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

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