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CULTIVATION OF THE MICROALGAE *CHLORELLA PYRENOIDOSA* USING THE PROCESSES OF BIOTECHNOLOGY

Cultivo de microalga *Chlorella pyrenoidosa* usando os processos da biotecnologia

Cultivo de microalga *Chlorella pyrenoidosa* usando los procesos de la Biotecnología

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Abstract: The results obtained here, show that the use of *Chlorella pyrenoidosa* microalgae and biotechnology, using the discontinuous process, presented satisfactory results. With this, the study of the microalga *Chlorella* sp. has proved to be important because it has a wealth of proteins, carbohydrates, amino acids, fatty acids, carotenoids, vitamins and minerals in its constitution, which may represent commercial importance. This research revealed the best results for obtaining a lipoprotein-rich biomass, taking into account three different culture media, calculations of cell concentration, cell productivity, to the content (%) of protein, lipid, carbohydrate and ash present in the microalgal biomass.

Keywords: *Chlorella pyrenoidosa*. Cell concentration. Cell productivity. Microalgal biomass. Content (%) (protein, lipid, carbohydrate and ash).

Resumo: Os resultados obtidos aqui, revelam que o uso da microalga *Chlorella pyrenoidosa* e da biotecnologia, com a utilização do processo descontínuo, se apresentaram como resultados satisfatórios. Com isto, o estudo da microalga *Chlorella* sp. mostrou-se importante, pois esta possui em sua constituição riqueza de proteínas, carboidratos, aminoácidos, ácidos graxos, carotenóides, vitaminas e minerais, o qual poderá representar importância comercial. Esta pesquisa revelou os melhores resultados estimados para a obtenção de uma biomassa rica em lipoproteína, levando-se em consideração a três diferentes meios de cultivo, aos cálculos de concentração celular, a produtividade celular, ao teor (%) de proteína, lipídio, carboidrato e cinza presentes na biomassa microalgal.

Palavras-chave: *Chlorella pyrenoidosa*. Concentração celular. Produtividade celular. Biomassa microalgal. Teor (%) (proteína**,** lipídio, carboidrato e cinza).

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Resumen: Los resultados obtenidos aquí, revelan que el uso de la microalga *Chlorella pyrenoidosa* y de la biotecnología, con la utilización del proceso discontinuo, se presentaron como resultados satisfactorios. Con esto, el estudio de la microalga *Chlorella* sp. se ha demostrado importante, pues ésta posee en su constitución riqueza de proteínas, carbohidratos, aminoácidos, ácidos grasos, carotenoides, vitaminas y minerales, el cual podrá representar importancia comercial. La investigación reveló los mejores resultados estimados para la obtención de una biomasa rica en lipoproteína, teniendo en cuenta a tres diferentes medios de cultivo, a los cálculos de concentración celular, a la productividad celular, al contenido (%) de proteína, lípido, carbohidrato y gris presentes en la biomasa microalgal.

Palabras clave: *Chlorella pyrenoidosa*. Concentración celular. Productividad celular. Biomasa microalgal. Contenido (%) (proteína, lípido, carbohidrato y gris).

INTRODUTION

Algae form the basis of the entire aquatic food chain (MÜLLER-FEUGA, 2000). On the cultivation of these algae, Brazil being a country with a long coast, bathed by tropical and subtropical waters, supposedly propitious for the growth of these aquatic organisms and with great wealth of species, including representatives of economic value with innumerable lagoons, numerous rivers (OLIVEIRA & MIRANDA, 1998, MORAES & JORDÃO, 2002). However, there is no tradition in the cultivation of these organisms and the national production is based on extractivism beginning in the 1940s (TOLEDO, 1953; OLIVEIRA, ALVEAL & ANDERSON, 2000). Another aspect is that care with the springs is not noticed, since many are polluted and extinct (MORAES & JORDÃO, 2002). However, there is still a wealth of biodiversity in aquatic environments, which could be cataloged, exploited and researched to obtain more resources, not only food, cosmetics, textiles, and others. The growing concern with environmental resources, so that future generations can enjoy not only as an economic source, but also of appreciation, education, and environmental preservation is essential, for the formation of a country with more quality and extended life expectancy. In Europe, for example, France stands out in the production of algae, and most of its research was carried out with macroalgae of red-vinaceous and brown color (SANGIARDI, 2010). In Portugal, marine macroalgae are responsible for an important primary production in the national territory, and brown algae have harvests described since the 14th century (PEREIRA, 1996).

Among them, microalgae, which represent numerous food products not only for aquatic organisms, but also for the human diet, serving as vitamin and protein supplements (BOROWITZKA, 1988 and 1999; LÜNING, 1990; RODRIGUES et al., 2010). These can present an important resource for the obtaining of natural products, possessing great economic potential for the food, pharmaceutical and cosmetic industries, for providing natural dyes, polyunsaturated fatty acids and abundant amounts of proteins (BECKER, 1994; HOBUSS et al., 2011). Spoehr & Milner (1949) were the first scientists to detail and describe the environmental conditions on microalgae, in relation to the lipid composition and chlorophyll content of the microalga *Chlorella* sp. and some diatoms.

In relation to these scientists, most of the sources of their research come from macroalgae, with few coming from microalgae, so it is essential to have more studies with microalgae. Thus, the two main microalgae that have stood out for commercial use are: *Chlorella* sp. (used in food) and *Dunaliella salina* for the production of β-carotene, but there are others that are used in aquaculture. These have specific culture characteristics depending on the species, for example, *Chlorella* sp. grows well in nutrient-rich media, whereas *D. salina* grows at very high salinity (BOROWITZKA, 1999).

The *Chlorella* sp. (about 45%), carbohydrates (around 20%), amino acids, fatty acids (around 20%), carotenoids, vitamins and minerals (around 10%) can to represent commercial importance (BENEMANN, 1990; HOEK, MANN & JAHNS, 1995; CHEN, ZHANG & GUO, 1996; BELASCO, 1997; TOMASELLI, 2002; TOMASELLI, 2003). A comparison between different species of microalgae was made by Lubtiz (1963), making an analogy between the protein content found in the egg, soybean and the reference standard cited

in the FAO/WHO (Food and Agriculture Organization / World Health Organization, 1973) it was observed that in *Chlorella pyrenoidosa*, among the 18 aminoacids studied, there were 17 of them. Thus, the cell wall of this microalga, whose thickness is equivalent to 5.5 µm and contains approximately 4% protein, 7% lipid, 69% α-cellulose, 16% hemicellulose, 0.3% gray calcium), corresponding to more than 39.2% of the dry weight of the whole cell (NORTHCOTE, GOULDING & HORNE, 1958).

Thus, the microalga *Chlorella pyrenoidosa* belonging to the Chlorophyta Division, whose taxonomic classification (Adapted from CHICK, 1903) was highlighted in this study:

Domain Eukaryotes
Phylum Chlorophyta
Class Trebouxiophyceae
Order Chlorellades
Family of Chlorellaceae
Genus *Chlorella*
Species *C. pyrenoidosa*

Moreover, the importance of studying the biotechnological processes applied to microalgal cultures, with the aim of obtaining natural compounds to be implanted in cosmetics, are being the subject of numerous researches and are an environmentally sustainable alternative (RENN DW, 1990; PLAZA, CIFUENTES & ILBANEZ, 2008; CARDOZO et al., 2007). Finally, algae-based products, in addition to having a high commercial value, are an important natural source for a wide range of valuable compounds including a diversity of pigments, which are used as natural dyes in cosmetics, food and the pharmaceutical industry, in this way the multi-use of algal inputs may direct benefits to health as a whole (KULSHRESHTHA & SINGH, 2013).

Thus, the secondary metabolites synthesized by these algae showed a broad spectrum of bioactivity (MANILAL et al., 2009; ARUNKUMAR, SIVAKUMAR & RENGASAMY, 2010). Microalgal extracts have the capacity to produce organic compounds, presenting several characteristics, for producing a series of bioactive molecules with effects, such as: antibiotics, anticancer, anti-inflammatory, antiviral, cholesterol reducers, enzymatic, antioxidants, photoprotectors, regeneration (due to the presence of copper) and blood (due to the presence of iron), among other examples (DERNER, OHSE & VILLELA, 2006; BOHNE & LINDEN, 2002; JENSEN et al., 2007; BERDILI et al., 2009).

However, the problem with the cultivation and commercialization of these microalgae is the need for closed, high-capital systems and the high cost due to the need for light (BOROWITZKA, 1999). For this reason, it is necessary to search for specific forms of crops for each species and to understand the physiology, so that a large amount of biomass can be obtained at a lower cost (FRANKLIN & FORSTER, 1997). Therefore, the use of biotechnological techniques (discontinuous and / or discontinuous fed processes) and the production of new, more sophisticated microalgal products may influence the development of more sophisticated pharmaceutical formulations in order to care for the skin.

MATERIAL AND METHODS

PREPARATION OF THE INOCULUM

C. pyrenoidosa was maintained in liquid standard medium. This was collected under aseptic conditions in laminar flow and inoculated in 10 mL of liquid medium in test tube for its growth. After 15 days, this material was used to inoculate 500 ml Erlenmeyer flasks with 250 ml sterile standard culture medium, the initial concentration of which was 200 $mg.L^{-1}$. The cultivation was intercalated between the two types of shakers (A and B). Erlenmeyer flasks were used during the experiment, after which the flasks had the following

conditions: 100 rpm.min^{-1}; minimum and maximum temperature, 25 ° C and 29 ° C, respectively; 72 µmol photons. m^{-2}.s^{-1} stirring (100 rpm / min); periodicity (12/12 h (L / E)); and culture medium (Bold Standard) associated with 7 mM $NaNO_3$ and 7 mM $(NH_4)_2SO_4$ (Adapted from ARAÚJO, 2015). After 6 to 8 days of cultivation, the biomass obtained was used as inoculum in Erlenmeyer flasks. The cell suspensions were filtered and washed with physiological solution for total withdrawal of the nitrate and resuspended in standard culture medium free of nitrogen source. For the final experiment, the *C. pyrenoidosa* suspension served as inoculum, in which cultures were carried out in 6000 mL erlenmeyer flasks, with a volume of 5000 mL of culture medium, whose initial concentration was 400 ± 0.02 mg. L^{-1} (adaptation of ARAÚJO, 2015).

MICROALGAL MATERIAL (ADAPTED FROM THE ARAÚJO, 2015)

In this work the microalgae *Chlorella pyrenoidosa* from discontinuous shaker cultivation (Erlenmeyer flasks (hermetically sealed)) with adequate light intensity and agitation was used.

In this work, this standard culture medium (**Table 1**) was used as sources of nitrogen used in inoculum preparation.

Table 1: Composition of the standard Bold medium for *Chlorella pyrenoidosa* UTEX 1663 (BISCHOFF & BOLD 1963; UTEX, 2011).

COMPONENTS	AMOUNT
$NaNO_3$	2,94 mM
K_2HPO_4	0,43 mM
KH_2PO_4	1,29 mM
NaCL	0,43 mM
$MgSO_4.7H_2O$	0,3 mM
$CaCL_2.2H_2O$	0,17 mM
* **Metal Solution (PIV)**	6 mL
****Mixture of vitamins**	1 mL

* **Metal Solution** (**PIV)** (mg.L^{-1}): 6 mL/L

Na_2EDTA, 750; $FeCl_3.6H_2O$, 97; $MnCl_2.4H_2O$, 41; $ZnCl_2$, 5;$CoCl_2.6H_2O$, 2; $Na_2MoO_4.2H_2O$, 4.

**** Mixture of vitamins** (mg/ 100 mL H_2O): 1 mL/L

Vitamin B_{12} (13,5 mg); Tiamina (2,5 mg) e Biotina (110 mg).

***** Micronutrient solution (**Chu)** (mg.L^{-1}): Na_2EDTA, 50; H_3BO_3, 618; $CuSO_4.5H_2O$,19,6; $ZnSO_4.7H_2O$,44; $CoCl_2.6H_2O$,20.

DETERMINATION OF CELLULAR CONCENTRATION

Three experiments were carried out with three different cultures, but from the same standard medium Bold (5mM $NaNO_3$ + 5mM $(NH_4)_2SO_4$; 7mM $NaNO_3$ + 7mM $(NH_4)_2SO_4$ and 10mM $NaNO_3$ + 10mM $(NH_4)_2SO_4$). The cellular concentration for *C. pyrenoidosa* was determined by withdrawing 5 ml of microalgae suspension throughout the experiment (8 days) via the test tube. In this way, optical density measurements were carried out using a FEMTO 700 PLUS spectrophotometer, whose reading wavelength was 686nm, and the following equation was used Growth rate (wet biomass) = (observed optical density / 0.0563) (R^2 = 0.9989) (DHULL et al., 2014).

DETERMINATION OF pH

The determination of pH in the culture of *C. pyrenoidosa* was carried out using the potentiometric method. The potentiometric device used was of the brand METTLER TOLEDO_PH2100e.

BIOMASS EVALUATION OBTAINED

The evaluations for both shaker cultivation and photobioreactor cultivation were employed using the following technique: at the end of *C. pyrenoidosa* cultures, the cell suspensions were centrifuged with three successive washes using distilled water for the withdrawal of the salt adsorbed by the cells after the biomass was subjected to drying at a temperature of approximately 55°C for 10 hours (adapted from the ARAÚJO, 2015). Subsequently, analyzes from this material were obtained for: proteins, lipids, ashes and carbohydrates.

DETERMINATION OF THE PROTEIN CONTENT OF BIOMASS *C. PYRENOIDOSA*

For the determination of total protein content in dry biomass, the classical KJELDHAL method (OFFICIAL METHODS OF ANALYSIS, 1984) was used. For this methodology, the defatted, dried and pulverized biomass resulting from the analysis of total lipids was used. The sample was digested in acid medium, with catalyst addition, in a digester block (350°C) until the material appeared clear. The sample was then directed to a nitrogen distiller. To this was added sodium hydroxide solution (60%) and recovery of the nitrogen in the ammoniacal form, in saturated solution of boric acid. Therefore, the resulting material obtained from this distillation process was titrated with standard 0.02 N HCl. Thus, the estimated protein percentage in the dried biomass could be calculated.

DETERMINATION OF THE LIPID CONTENT OF BIOMASS *C. PYRENOIDOSA*

The extraction of the total lipid fraction was performed using organic solvent (OLGUÍN et al., 2001), according to the methodology described below: The dry sample: crushed in gral, weighed approximately 1g from the biomasses obtained in shaker cultures, for *C. pyrenoidosa*. The biomass was then transferred to the Soxhlet beaker or cartridge and finally coupled to the Soxhlet continuous extractor so that it was extracted with a mixture of chloroform-methanol (2: 1) (v / v) by a period of 4 hours. At this time, the solvent present at the reflux containing the material extracted from the Soxhlet, was clear.

After this time of extraction, the round bottom flask containing the extracted material was taken to the rotavaporator or rotary evaporator, to extract all the solvent chloroform-methanol (2: 1) (v / v), which was placed in the beginning of the extraction technique (KHOR & CHAN, 1985). With this, inside the round bottom flask all concentrated lipid material extracted in this technique was obtained. This material consists of several components: fatty acids (polyunsaturated (contain two or more unsaturations), monounsaturated (contains a double bond per molecule), saturated (contains double bonds)), triglycerides, phospholipids, carotenoids, photosynthetic pigments, steroids and hydrocarbons, referred to as total lipid fraction (FERRAZ, 1986).

Thus, the determination of the lipid fraction was performed through the mass of the round bottom flask after this extraction technique (round bottom flask cooled to room temperature cooled in desiccator with the lipid material adsorbed). Thus, a mathematical operation (subtraction) was performed taking into account the round bottom balloons (previously heavy and empty), so that the estimate of the percentage in relation to all the existing components in the sample of the lipid material is thus revealed.

DETERMINATION OF ASH CONTENT FOR *C. PYRENOIDOSA*

The ash content was determined by adapting the method described by the Brazilian Pharmacopoeia in 2010 (FARMACOPÉIA BRASILEIRA, 2010). The dried and pulverized biomass of *C. pyrenoidosa* was

transferred to the porcelain crucible (previously calcined, cooled to room temperature and weighed). The sample was then uniformly distributed in the crucible, and was then directed to the muffle furnace. The temperature was gradually raised to 600°C for approximately 4 hours until the ashes were fully obtained.

DETERMINATION OF TOTAL CARBOHYDRATE CONTENT FOR *C. PYRENOIDOSA* (ARAÚJO, 2015)

The total carbohydrates content was determined by difference between the percentages of the components analyzed here: total proteins, total lipids and total ashes.

RESULTS AND DISCUSSION

Considering that the cellular growth and the biomass composition of the microalgae are influenced by both the nitrogen source and the concentration of the microalgae (BOUSSIBA & RICHMOND, 1980; NAES, UTKILEN & POST, 1988; STANCA & POPOVIC, 1996; SASSANO et al., 2010). In this study, a number of sources of nitrogen, such as ammonium sulfate (SOLETTO et al., 2005) and sodium nitrate (COSTA et al., 2001) shaker, for *C. pyrenoidosa*. Thus, in the assays of *C. pyrenoidosa*, carried out in shaker shakers, with 6000 mL Erlenmeyer flasks containing 5000 mL of medium, the initial cell concentration was approximately 400 $mg.L^{-1}$.

The independent variable was the microalgae (*C. pyrenoidosa*). The cultivation conditions for pH and light intensities for this microalga were based on information from the literature used and through the results propagated and obtained, also by the scientist Araújo, 2015; and the dependent variables (responses, results), which were measured in this study: cell growth; cell productivity; protein content, carbohydrates, ashes and lipids.

Thus, all the experiments of this research were evaluated through analysis of variance (one-way ANOVA), using the Tukey tests applied with 95% confidence interval, using the program Statistic 11.0 and obtaining the following evaluations : the cell growth in relation to the culture time in the shaker for *C. pyrenoidosa*; the cell growth in relation to the different culture conditions (feed source, different culture media) for *C. pyrenoidosa*; the percentages of proteins, lipids, ashes and carbohydrates, for each distinct culture medium studied here.

The dependent variables, in relation to the dry weight of shaker for *C. pyrenoidosa*, such as: maximum cell concentration (Xm), cellular productivity (Px), protein content (Tprot), lipid (Tlip), carbohydrates and total ashes. These were considered essential to obtain the best result regarding the culture medium used for the biomass production of this microalgae under study.

The composition of the culture medium and the biomass composition of *C. pyrenoidosa* in shaker are reported through the best condition of nitrogen supply characterized by discontinuous cultivation process.

Paoletti, Pushparaj & Tomaselli (1975) and Schlösser (1982) described the nutritional elements essential for the cultivation of *A.* (*Spirulina*) *platensis*, which come from nitrogen and can be supplied through inorganic salts such as potassium nitrates and or of sodium. In this research the sodium nitrate mentioned by these scientists was used, and the *C. pyrenoidosa* studied here used the nitrates from this salt, consuming them well. According to Araújo, 2015 was observed for *A.* (*Spirulina*) *platensis* that it grew well only with sodium nitrate, and in relation to *C. vulgaris* there was a more favorable growth with the mixture of this salt with another, in the case of this study, ammonium sulfate; the same occurred in this study, for *C. pyrenoidosa*, however, only the best results obtained through three different culture media were presented here.

Miller & Colman (1980) reported in their studies that cyanobacteria preferentially assimilate the bicarbonate form, and that the pH value of the culture medium is influenced by the proportion of this compound. Binaghi et al. (2003) mentioned the carbon source used in the form of bicarbonate and / or carbonate during the cultivation of *A.* (*Spirulina*) *platensis*, in which the cells preferentially consume the

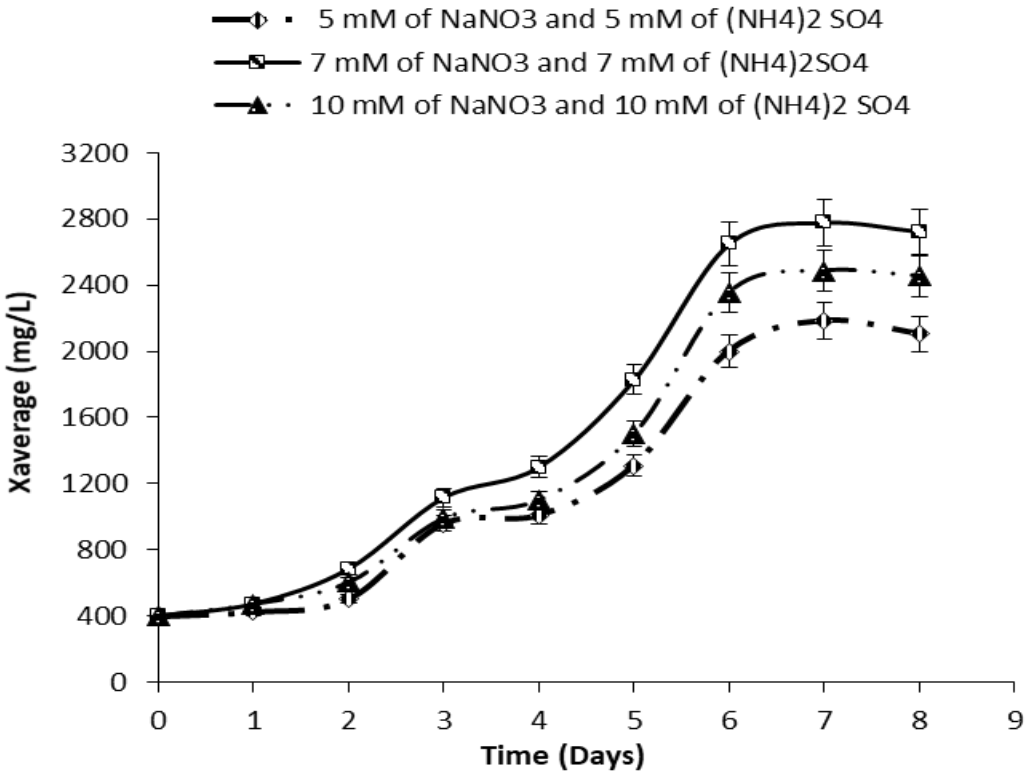
bicarbonate and release the carbonate into the culture medium. In the literature (BECKER, 1994; ANDERSEN, 2005; SOLETTO et al., 2008; ALABI, TAMPIER & BIBEAU, 2009; FERREIRA et al., 2010; MATSUDO et al., 2011; RAMANATHAN & FENG, 2008; among others) it is explained that the imbalance (bicarbonate / carbonate) is directed to an increase in pH, which can be avoided by supplying carbon dioxide to the medium, but it is essential to keep the pH constant at an ideal value for the culture, which in the case for *C. pyrenoidosa* was the same as that used by *C. vulgaris* maintained at values of 7.5 ± 0.5, also with use of carbon dioxide gas. Its use for pH control and as a carbon source shows the importance of its use on an industrial scale, which would contribute to the reduction of the greenhouse effect, as reported by IPCC (Intergovernmental Panel on Climate Change) in 2007.

In this research, the process used was the discontinuous shaker, and the cell growth rates were correlated with each other. In this way, it was directed to the formation of biomass with higher content of proteins and lipids in them, and these biomasses were used in the form of dry biomass.

EXPERIMENT PERFORMED IN SHAKER FOR *C. PYRENOIDOSA* TO VERIFY CELL GROWTH

The results of the averages values of *C. pyrenoidosa* triplicates revealed that the medium containing 7 mM $NaNO_3$ + 7 mM $(NH_4)_2 SO_4$ (discontinuous process) was the one that obtained the highest microalgal growth (**Figure 1**).

Figure 1: Results of the tests performed with the Bold medium, using the shaker.



The results of the triplicate averages of the *C. pyrenoidosa* experiment performed in the shaker (**Figure 1**) revealed that the cultures receiving 5 mM $NaNO_3$ + 5 mM $(NH_4)_2SO_4$ were the ones with the lowest values of cell growth. The results of the three distinct concentrations for mean maximum cell growth (Xm) and average cell productivity (Px) respectively were: 5 mM $NaNO_3$ and 5 mM $(NH_4)_2SO_4$ (2283 ± 0.5 $mg.L^{-1}$ and 256.37 ± 0.2 $mg.L^{-1}d^{-1}$); 7 mM $NaNO_3$ and 7 mM $(NH4)_2SO_4$ (2778 ± 0.3 $mg.L^{-1}$ and 339.8 ± 0.1 $mg.L^{-1}d^{-1}$); and 10 mM $NaNO_3$ and 10 mM $(NH_4)_2SO_4$ (2536 ± 0.4 $mg.L^{-1}$ and 299.13 ± 0.2 $mg.L^{-1}d^{-1}$). The best cell growth medium for *C. pyrenoidosa* was Bold enriched with 7 mM $NaNO_3$ and 7 mM $(NH_4)_2SO_4$, which was then performed calculations of protein, carbohydrate, ash and lipid contents. Also, lipids were identified and quantified. **Table 2** shows the highest growth, among the culture media tested and studied here.

According to Araújo (2015) the same genus *Chlorella*, but the different species *C. vulgaris* showed a better growth when grown in Bold medium enriched with (5 mM of $(NH_4)_2SO_4$ + 5 mM of $NaNO_3$) in relation to the other culture media. Since this current research required 2 mM of each salt ($(NH_4)_2SO_4$ and $NaNO_3$), for *C. pyrenoidosa* to reach its highest cell growth, when compared to the other two media already mentioned above.

Table 2: Cell growth expressed in $mg.L^{-1}$

Photosynthetic micro-organism	***C. pyrenoidosa***
Days	**Bold+7 mM of $NaNO_3$ and 7 mM of $(NH_4)_2SO_4$ [a]**
0	400±0,2 A
1	473±0,1 A
2	685±0,2 B
3	1115,5±0,1 C
4	1300,1±0,1 D
5	1827,8,8±0,3 E
6	2750±0,1 F
7	2778,3±0,1 F
8	2724,5± 0,3 F

[a] Average values (performed in triplicate)

* Different letters in the same column represent statistically different values.

DETERMINATION OF PROTEIN, LIPID, ASH AND CARBOHYDRATE FOR *C. PYRENOIDOSA* IN SHAKER

The averages (in triplicates) of the results were presented, in relation to the different media studied. These presented different levels (%) of the compositions related to the biomasses of *C. pyrenoidosa*, in cultures realized in shakeres (**Table 3**). According to Chisti (2007) in his studies with microalgae, which presented a high lipid content in dry biomass ranging from 30% to 70%, which did not corroborate with this current research, since *C. pyrenoidosa* presented approximately 24% lipid content in dry biomass. According to Becker (1994) the microalgae *C. pyrenoidosa* presented 57% protein, 2% lipid and 26% carbohydrate, which did not corroborate with this current research, as it is noted in the table below.

Table 3: Content of the final means (%) of the components, in relation to obtaining the highest growth rate through the shaker experiments (discontinuous process), from the dry biomass of *C. pyrenoidosa* (CP).

Photosynthetic micro-organism	***C. pyrenoidosa***
Cultivation medium	Bold+7 mM of $NaNO_3$ and 7mM of $(NH_4)_2SO_4$
Content (Results (%))	
% Protein[a]	52,65 ± 0,01A
% Lipid[a]	23,85 ± 0,03B
% Ashes[a]	2,12 ± 0,03C
% Carbohydrates[a]	21,42 ± 0,01B

[a]Average values. Different letters in the same column represent statistically different values.

CONCLUSION

This research made use of biotechnology, involving the discontinuous process (test for *C. pyrenoidosa*, performed in shaker, and with the best result of this comparing three types of microalgae culture among themselves).

In shaker experiments, the best culture medium for *C. pyrenoidosa* was Bold medium enriched with 7 mM $(NH_4)_2$ SO_4 and 7 mM $NaNO_3$. Thus, the following estimated results were obtained: maximum cell concentration (Xm) of 2778 ± 0.3 $mg.L^{-1}$, cell productivity (Px) of 339.8 ± 0.1 $mg.L^{-1}d^{-1}$, protein contents (Tprot) = 52.65%, lipids (Tlip) = 23.81%, carbohydrates (Tcarb) = 21.42% and ashes (Tcz) = 2.12%.

In addition, in the experiments performed in photobioreactor, the best culture medium for *C. pyrenoidosa* was Bold medium (7mM $NaNO_3$ and 7mM $(NH_4)_2SO_4$) obtained from shaker tests. With this, it was possible to obtain the highest cell growth or maximal cell concentration, with Xm reaching the value of 2778 ± 0.3 $mg.L^{-1}$ (batch process) on the seventh day of culture, and whose productivity in cells Px) was 339.8 ± 0.1 $mg.L^{-1}.d^{-1}$.

Thus, this research revealed the best results estimated to obtain a biomass rich in lipoprotein. The results obtained in this research, using microalgae *C. pyrenoidosa* and biotechnology, using the batch process, presented satisfactory results.

Therefore, obtaining a natural raw material (quality and safe) will depend on, according to Araújo, 2015: i) many parameters (factors): some (for shaker) (such as temperature, luminous intensity and tests with different nutrient sources, pH was maintained in a "manual"), and these were carried out in this current research. However, other factors (essential), due to this research being extensive, will not be employed here. Unfortunately, they will only be cited in order to favor learning, such as seasonality or seasonal variation (date of collection) and the circadian cycle or circadian rhythm (time of collection), among others. Thus, the use of all parameters in obtaining a natural quality and safe raw material, coupled with the most modern, correct and appropriate analysis techniques, will make the whole process probably reproducible and stable.

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