e-ISSN: 0974-4614
p-ISSN: 0972-0448

Productive efficiency of chlorella vulgaris in nutritional and energy source conditions for use in bioremediation processes

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Received: 24.02.2024 Revised: 16.04.2024 Accepted: 18.05.2024

ABSTRACT
The aim of the present study was to evaluate in vitro the production of proteins from the microalga Chlorella vulgaris by enriching the culture medium with different organic nitrogen sources and light types. The selection of organic nitrogen sources with which the medium was enriched was carried out and the growth of the culture, microalgal biomass productivity and Chlorella vulgaris protein production were evaluated in different types of light (sunlight, red, white and blue). The samples were monitored for 35 days maintaining the temperature range of 20 to 25 °. The results indicate that the concentration, the type of light and the combination of both have an impact on the growth and protein production of the microalga Chlorella vulgaris. The highest production of wet protein (PH-BH), from the microalgal biomass of Chlorella vulgaris was obtained when molasses was used as a protein source in the presence of wavelength (white light and sunlight) and lower for wavelength (blue light).

Keywords: Microalgae, nitrogen source, energy source, bioaugmentation

1. INTRODUCTION

The use of the microalgae Chlorella vulgaris has attracted attention in the field of biotechnology, becoming an interesting source of biomass for the biodiesel production sector (Cléber et al., 2006). Different investigations have reported an increase in the capacity of nutrient assimilation and in its growth, which has led to the suggestion that the microalga Chlorella vulgaris be used in the development of bioremediation processes, due to its ease of adaptation and its accelerated growth in the environment (Brennan et al., 2010).Chlorella Vulgaris are unicellular photosynthetic organisms that use light and carbon dioxide, with greater photosynthetic efficiency than plants, for the production of biomass. According to Metsoviti et al., (2019); Cartagena and Malo, (2017), they can be grown in various media, in organic or inorganic substrates for their growth. The factors that influence the growth of microalgae are similar, however, each species and subspecies of microalgae has its own characteristics regarding its optimal growth conditions and its productivity is determined mainly by the pH of the medium, salinity, availability and concentration of nutrients, intensity and type of light, cell density of the culture, temperature and contamination (Cartagena and Malo, 2017; Moreno et al., 2012) .As proposed by Parra et al., (2019) ,The supply of nutrients in adequate quantities is essential for obtaining good cell growth of microalgae, which in turn plays a fundamental role in the production of different metabolites, such as lipids, proteins and pigments .

The studies and evidence shown by Becker, (2007); Núñez and Ortega, (2016) , are that Chlorella vulgarisIt is a unicellular freshwater green algae with a spherical morphology and a protein content that can vary approximately from 50-55%, with 5% chlorophyll and a large number of compounds such as vitamins and carotenes; in addition, it contains essential amino acids for the human body and is a source of high-quality protein according to the Food and Agriculture Organization of the United Nations (FAO).

Based on the importance of the microalga Chlorella vulgaris as a biotechnological resource, the strategy proposed was to evaluate in vitro the capacity of this microalgae to grow and produce biomass in the presence of different sources of nitrogen using raw materials from the region and to grow through photosynthetic processes using four energy sources.

2. MATERIALS AND METHODS

Cell. Biomass of the microalga Chlorella vulgaris was used (CVLINM 99%) was obtained from the germplasm bank collection of the Microbiological Research Laboratory of the University of Sucre. The cells were maintained in Nutrifoliar culture medium (Colinagro 4.0), containing 200 g/l of total nitrogen, 100 g/l of phosphorus and nutrients such as K, Mg, S, Cl, Fe, Cu, Zn, Mn and Mo (Vitola Romero et al., 2021); This medium was diluted to 2% v/v in distilled water and sterilized in an autoclave.

Nitrogen sources. Nitrogen sources were selected taking into account variables such as availability, cost, protein content and use of agro-industrial waste. Based on this analysis, black sugar cane honey, whey and Enterolobium seeds were chosen. Cyclocarpum (earwort) as organic sources of nitrogen.
For molasses, concentrations of 1.0 g/L, 2.0 g/L and 3.0 g/L were used, taking into account the methodology described by (Michele R. Andrade; Jorge Costa, 2007), for whey, concentrations of 1.0 g/L, 1.5 g/L and 2.0 g/L were taken according to the study by (Murcia and Parra, 2018). Enterolobium seeds cyclocarpum (earwort) preliminary tests were carried out and the concentrations 1.0 g/L, 2.0 g/L and 4.0 g/L were taken because there was no reference data.
Growth curves. For the growth curve, 1-litre phycoreactor were used with a working volume of 500 ml of 2% v/v culture medium and sterilized distilled water, a $1 \cdot 10^6$celulasinitial inoculum concentration, enriched with the different organic nitrogen sources. The growth of the microalgae biomass was analyzed over a period of 35 days using a Spectroquant Pharo 300 spectrophotometer at a wavelength of 647 nm and compared with the control test of the microalgae with the culture medium.
Energy source. Sunlight, white light, blue light and red light were used as energy sources.

3. RESULTS AND DISCUSSION

Figure 1 describes the growth behavior of Chlorella vulgaris in the presence of four energy sources (sunlight, white, blue and red light). The graph shows that it showed greater growth in the presence of sunlight, followed by white light and less for red light.

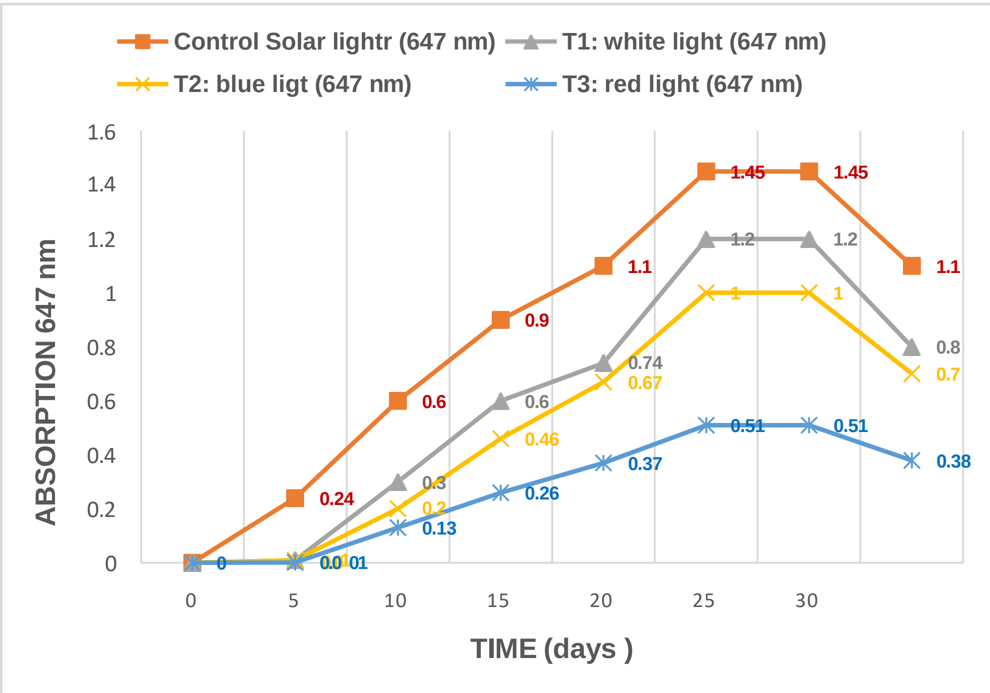

Figure 1. Chlorella growth curve vulgaris into four different energy sources.

The results of the Tukey test regarding the growth of microalgal biomass of Chlorella vulgaris as a function of carbon source and type of light, as indicated in table 1. The results obtained show that there is a significant difference between the parameters wet protein (PH-BH); dry protein (PB-BS); wet yield; dry biomass and productivity with respect to the type of light and the carbon source used (black honey from sugar cane, whey and oyster (legume seed).

Table 1. In vitro production of wet matter, dry weight, biomass and productivity of Chlorella vulgaris in the presence of different light sources and nitrogen.

Parameters	**Type of light**	**Source of nitrogen**		
		Black sugar cane honey	**Whey**	**Legume seed**
Wet protein (PH-BH)	Sunlight	57.1	52.1	53.1
	White	56.3	49.3	52.3
	Blue	50.2	48.2	47.2
	Grid	52.2	52.5	52.6
Dry protein (PH-PS)	Sunlight	62.1	62.1	56.1
	White	61.6	57.6	54.6
	Blue	53.9	53.2	50.9
	Grid	57.5	56.5	55.5
Dry yield g/L	Sunlight	23	2.9	6.3
	White	2.2	1.8	6.2
	Blue	1.1	0.98	3.5
	Grid	0.98	2.5	5.2
Dry biomass g/L	Sunlight	1.02	1.02	0.9
	White	0.98	0.09	0.25
	Blue	0.06	0.03	0.12
	Grid	0.04	0.09	0.202
Productivity g/L/d	Sunlight	0.09	0.034	0.056
	White	0.06	0.04	0.011
	Blue	0.03	0.02	0.06
	Grid	0.02 b	0.03	0.1

The highest wet protein production (PH-BH) of the microalgal biomass of Chlorella vulgaris was obtained when molasses was used as a protein source in the presence of wavelengths (white light and sunlight) and lower for wavelengths (blue light). Regarding the dry yield of the microalgal biomass, the highest value was presented in sunlight and white light and lower for blue light when the growth medium was supplemented with a protein source from whey. Regarding the dry matter content for the microalgal biomass, the highest values in content were recorded in the growth of the microalga in the presence of a protein source from whey when the type of light used was sunlight and white light and lower for blue light. Finally, higher productivity of microalgal biomass was observed in the medium supplemented with black sugar cane molasses in the presence of sunlight and white light and lower for blue and red light, respectively.

According to Lewandowski et al., (2018), microalgae are a diverse group of unicellular microorganisms, with around 300,000 different species existing on the planet, of which only around 40,000 have been described. This group includes cyanobacteria (prokaryotes), as well as eukaryotic microalgae species capable of growing in diverse environments (Lewandowski et al., 2018). Most are photoautotrophic, meaning that light is their energy source, while CO2 $_{\text{is}}$ their carbon source.

According to Gamarra Santos (2019), the photosynthesis carried out by microalgae is a very important process, since marine phytoplankton produces approximately 50% of the oxygen in the atmosphere. Microalgae use photosynthetically active radiation or PAR (Photosynthetically Active Radiation), which is in the wavelength range of 400 to 700 nm. Photosynthesis converts the absorbed light energy into chemical energy, usable for cell development and growth, with NADPH and ATP being the molecules in which this chemical energy is stored. Oxygenic photosynthesis can be expressed as an oxidation-reduction reaction promoted by light energy, in which water and CO_2 are converted into oxygen and carbohydrates.

Photosynthetic pigments present in photosynthetic organisms are of great importance, being the basis of life on earth according to Reol (2003). The primary pigments have the function of capturing light energy. The primary pigment in all algae is chlorophyll a, which is associated with proteins of the reaction center and the antenna pigments of photosystem I (PSI) and photosystem II (PSII), and can be classified according to whether they are prokaryotic photosynthetic organisms (bacteriochlorophyll a) or eukaryotic (chlorophyll a).

Chlorella vulgaris has chlorophyll b that acts as a light harvester, in addition to transferring light energy to chlorophyll a (Mena Bolaños, 2019). Chlorophyll a, together with chlorophyll b, are associated with the external energy harvesting complexes of PSI and PSII called LHCI and LHCII, respectively (Nelson and Yocum , 2006). These are accompanied by other accessory pigments (carotenoids), whose function is to broaden the absorption spectrum of the primary pigments, in addition to protecting against an excessive amount of light, dissipating the

excess absorbed energy, so that it is not harmful (Gamarra Santos, 2019). Carotenoids are a class of pigments that are produced in two main types, the hydrocarbon class (carotenes) and the oxygenated class (xanthophyll), which have a strong antioxidant property.
biomass needs to have different types of nutrients available for its growth. Macronutrients are part of the cell structure and are needed in higher percentages (N, P, C, H, S). Micronutrients are elements that the cell needs in small quantities and that usually form part of enzymes (Fe, Mn, Cu or Mo) (Rodríguez González and Serrano Luna, 2012). The most important nutrient for microalgae is carbon, followed by nitrogen, which is incorporated as nitrate ($NO3^-$) or as ammonium ($NH4^+$). Phosphorus is essential in many cellular processes, such as the formation of nucleic acids and energy transfer. In addition, it appears in important metabolic intermediates and in structural molecules such as phospholipids.
The media enriched with black sugar cane honey and whey presented the highest percentage of protein in wet and dry biomass, using sunlight and white light as energy sources and less for blue and red light, which is similar to the results found by Coca et al., (2015) , who indicate that by enriching the medium with a concentration of [ ] 2.0 g/L of beet vinasse (by-product of molasses fermentation) the highest protein content (72%) is produced from the microalgae Spirulina platensis using fluorescent lamps (Osram F40CW, 40 W) at the lowest light intensity and Schlösser medium for a culture time of 20 days, the protein percentage of this research was even higher, however it must be taken into account that for the experiment they used another type of microalgae, periods of 16 h of light and 8 h of darkness and tubular bioreactors which was favorable for this study .

4. CONCLUSION

Enriching culture media of the microalgae Chlorella vulgaris with organic nitrogen sources is beneficial, as it produces an increase in crude protein content, dry weight percentage, yield and productivity. The results indicate that the concentration, type of light and the combination of both have an impact on the growth and protein production of the microalga Chlorella vulgaris. Media enriched with black sugar cane honey, followed by whey and legume seeds, presented the highest percentages of protein in wet and dry biomass, using sunlight and white light as energy sources.

5. ACKNOWLEDGEMENTS

The authors would like to thank the Microbiological Research Laboratory of the University of Sucre.

6. Author contribution

Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. Conflict of interest

All the authors of the manuscript declare that they have no conflict of interest.

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