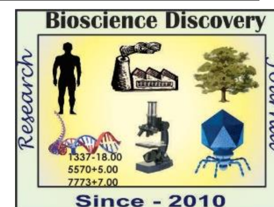
Bioscience Discovery, 15(4):42-45, October - 2024

Open Access, Peer Reviewed, Referred Research Journal
© RUT Printer and Publisher
Print & Online available on https://jbsd.in

ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)



Research Article

Cultivation of *Chlorella pyrenoidosa* and *Spirulina subsalsa* using a photobioreactor: A preliminary study

Sruthi P. Pillai[1], Rutwik Thengodkar[2], Deepali Dande[3] and Sangeeta A. Godbole[4]*

[1,4*]Department of Botany, Jai Hind College, Churchgate, Mumbai, India-400020
[2,3] R&D, Cyanohealth Research Division, Mumbai, India-400091

Article Info

Received: 12-06-2024,
Revised: 01-07-2024,
Accepted: 22-08-2024

Keywords: *Chlorella pyrenoidosa*, Microalgae, phototrophic cultivation, *Spirulina subsalsa*, standardized conditions

Abstract

Microalgae are well-known as a potential source for valuable bioactive metabolites. Among these compounds, carotenoids have received industrial demand for their health promoting functions, increasing the significance of microalgal cultivation known for their active production. Standardization of cultivation methods as per algal species under study is the preliminary and an essential step for any algal research. Though several studies have explored carotenoid production in different microalgae, there is limited data on the profiles of carotenoid production in the green algae *Chlorella pyrenoidosa* and the blue-green algae *Spirulina subsalsa*. This study aimed to cultivate *Chlorella pyrenoidosa* (NCIM-2738) and *Spirulina subsalsa* (NCIM-5143) phototrophically using a photobioreactor under standardized cultivation conditions (temperature (28°C-35°C), relative humidity (50%-60%), and light intensity (2.5-3.5 klx)) and to prepare inoculum for subsequent experiments involving carotenoid extraction. *C. pyrenoidosa* and *S. subsalsa* were cultivated in modified Bold's Basal Medium (BBM) and Zarrouk's culture medium, respectively under sterile conditions. A photobioreactor was developed using acrylic sheet and an aluminum sheet base to provide optimal cultivation conditions. An agitator system was developed to support efficient mixing of cultures. Natural white light LED strips provided illumination, with a light and dark photoperiod set to (10/14 hours). Cultures were scaled up every two weeks and final volumes were maintained to 4 liters for *C. pyrenoidosa* and 2 liters for *S. subsalsa*. The cultivation of *Chlorella pyrenoidosa* and *Spirulina subsalsa* were successfully achieved providing its use as culture inoculums for further experimentation.

INTRODUCTION

Migroalgae produce and secrete valuable metabolites in response to stressful conditions like high or low temperature, photo-oxidation, ultraviolet radiation, high salinity, and osmotic pressure (Ren *et al.,* 2021). They are protective in function against reactive oxygen species (ROS) which are known to damage human health (Cao & Prior, 1998; Halliwell & Gutteridge, 1990; Hu *et al.,* 2007). Among all the metabolites, carotenoids are found to have several health benefits (Park *et al.,* 2018; Rao & Rao, 2007; Stahl & Sies, 2005; Tapiero *et al.,* 2004). Carotenoids, including β-carotene, lycopene, lutein, canthaxanthin, and astaxanthin are highly valued in the food industry due to their antioxidant properties (Gong & Bassi, 2016).

While numerous studies have investigated carotenoid production in various microalgae, there is limited data regarding the specific profiles of carotenoid production in the green algae *Chlorella pyrenoidosa* and the blue-green algae *Spirulina subsalsa*. Standardizing cultivation methods based on the algae species being studied is a fundamental primary step in any research involving algae.

Cultivation of algae can be achieved using open pond systems or closed photobioreactor systems. Photobioreactors are found to have more advantages than open ponds such as higher cell concentrations, reduced carbon dioxide loss and water evaporation, minimized contamination, and enhanced production of bio compounds (Soni *et al.,* 2017). However, closed photobioreactors (PBRs) and agitator systems can be costly, highlighting the need for a cost-effective substitute to achieve similar functionalities.

The present study focusses on exploring the cultivation of two microalgal species, *Chlorella pyrenoidosa* and *Spirulina subsalsa* known to contain various important metabolites, under standardized phototrophic conditions suggested by previous studies for establishing the groundwork for future experiments like carotenoid extraction (Coronado-Reyes *et al.,* 2020; Fagiri *et al.,* 2013; Hu *et al.,* 2007; Perera *et al.,* 2023; Ramlee *et al.,* 2021; Sharma *et al.,* 2012; Soni *et al.,* 2019). An attempt was made to construct a simple photobioreactor and implement an agitator system to provide standard growth conditions to achieve algal cultivation under a controlled environment (temperature (28°C-35°C), relative humidity (50%-60%), and light intensity (2.5-3.5 klx)).

MATERIALS AND METHODS

Procurement of Algal Strains

Spirulina subsalsa NCIM-5143 and *Chlorella pyrenoidosa* NCIM-2738 agar slopes were obtained from National Chemical Laboratory (NCL), culture collection center, located in Pune, India.

Morphological analysis of microalgal samples

The algal strains were examined under a light microscope at 10x and 40x magnification to verify their morphological characteristics and confirm their identification.

Photobioreactor Construction

A photobioreactor of size dimensions 33cm width x 47cm height x 43cm breadth was built using 3mm thick acrylic sheets. The acrylic sheets used were durable and transparent. This helped the algal cultures to receive light transmission required for photosynthesis and could be maintained for longer time. Additionally, an aluminium sheet was placed at the base of the photobioreactor on the inner side on account of its properties of stability and heat dissipation. Stock cultures of both algae were maintained in this photobioreactor under optimal growth conditions. Cultures were uniformly illuminated using 4 LED strips (each 5 meter in length) and interconnected to each other in series. The photobioreactor was placed in a dust free environment for experimental purposes. Physical parameters like temperature, relative humidity and light intensity were maintained and recorded daily, in the morning and evening, using a thermometer, humidity meter, and lux meter (Lutron LX-101A), respectively. For smaller volumes the cultures were agitated manually. However, with increase in culture volumes to 2 litres and 4 litres an affordable agitator system was developed for uniform agitation using a silicon bottle brush and a motor. A speed of 60 rpm was maintained to avoid cell damage as well as to ensure proper mixing. This avoided clumping of large filaments especially in case of *Spirulina subsalsa*, and avoided settling of cells at the base of culture flask in case of *Chlorella pyrenoidosa*

Selection of cultivation media

Chlorella pyrenoidosa was cultivated in the photobioreactor constructed as above using Modified BBM (Mubarak *et al.,* 2020). *Spirulina subsalsa* was cultivated similarly using Zarrouk's culture medium (C, 1966). Both culture media were allowed to store overnight to allow precipitation, if any, which was filtered off before algal inoculations. However, as the cultures could not survive in larger media volume and hence smaller volumes (20 ml) of culture media were used and this resulted in proper growth of algae.

Inoculum preparation, Scaling up and maintenance of cultures:

A loopful of *Chlorella pyrenoidosa* and *Spirulina subsalsa* sample from the NCIM agar slopes were transferred to freshly prepared sterilized 20 mL modified Bold's basal medium and modified Zarrouk's medium, respectively using 100 mL sterile conical flasks. Cultures were illuminated for 10/14 hour light /dark cycle at temperature (28°C-35°C), relative humidity (50%-60%), and light intensity (2.5-3.5 klx) (Coronado-Reyes *et al.,* 2020; Fagiri *et al.,* 2013; Ramlee *et al.,* 2021; Sharma *et al.,* 2012; Soni *et al.,* 2019). Cultures were manually agitated intermittently, at least twice a day, throughout the cultivation span. This avoided clumping of cultures and accelerated the growth process. Within the span of two weeks, *Chlorella pyrenoidosa* and *Spirulina subsalsa* showed growth in their respective media.

The cultures were maintained using a combination of fed-batch as well as continuous culture technique. The initial 20ml culture inoculums were slowly scaled up to a final volume of 4 litres for *Chlorella pyrenoidosa* and 2 litres for *Spirulina subsalsa* and maintained as such for further experiments. Both algal cultures were maintained on agar slants prepared using the same media composition used for each algal cultivation and additionally containing agar at a concentration of 1% (w/v). Agar slants could

sustain the algae for a period of one month under same growth conditions.

RESULTS AND DISCUSSION

Algal growth in Photobioreactor

The photobioreactor setup helped to achieve successful cultivation of *Chlorella pyrenoidosa* (NCIM-2738) and *Spirulina subsalsa* (NCIM-5143) and maintain standard cultivation conditions. Regular monitoring of temperature, humidity, and light intensity ensured consistent growth conditions.

Selection of Cultivation Media and Maintenance of Cultures

The main challenge in cultivation experiments was media precipitation. as both the medium showed precipitation during preparation. Several trial and error attempts were made to prevent media precipitation. While precipitation was successfully avoided in modified BBM, Zarrouk's culture medium continued to show precipitation, where $FeSO_4.7H_2O$ was identified as the precipitating agent.

Despite these challenges, both Modified BBM and Zarrouk's culture medium were effectively utilized for inoculum preparation and subsequent cultivation of *Chlorella pyrenoidosa* and *Spirulina subsalsa,* respectively. *Spirulina subsalsa* showed successful growth and reached the stationary phase within two weeks when grown in 3-liter glass bottles (containing 2 litres of culture medium) due to the large medium requirement by *Spirulina. Chlorella pyrenoidosa* cultures showed slower but sustained growth and did not reach stationary phase within 2 weeks. Hence, these cultures were maintained in higher culture volumes of 4 litres contained in 5-liter glass bottles. This ensured reduced maintenance for *Chlorella pyrenoidosa* cultures due to less frequent addition of fresh medium. The agitator system provided uniform mixing of cultures. The cultures were also able to sustain in the agar slants prepared from liquid cultures.

Discussion

This study attempted cultivation of *Chlorella pyrenoidosa* and *Spirulina subsalsa* inside a photobioreactor under standard phototrophic conditions used for other *Chlorella* and *Spirulina* species. Cultivation was successful in both the media. A combination of fed-batch as well as continuous culture technique were employed for maintenance of both algal cultures. *Spirulina*, reached the stationary phase within two weeks as compared to *Chlorella pyrenoidosa* cultures which required 4 weeks to reach stationary phase. These growth observations aligns with the previous studies of *Chlorella pyrenoidosa* and other *Spirulina* species (Balaji *et al.,* 2015; Ismaiel *et al.,* 2016; Rana *et al.,* 2020). These growth observations align with the previous studies of *Chlorella pyrenoidosa* and other *Spirulina* species (Balaji *et al.,* 2015; Ismaiel *et al.,* 2016; Rana *et al.,* 2020). The present study offers valuable information regarding cultivation of industrially important *Chlorella pyrenoidosa* and *Spirulina subsalsa,* which is also a preliminary step for conducting any further experiments on pigment analysis and extraction for industrial use from these algal species.

Figure 1. Steps involved in construction of a photobioreactor: a) Construction of a photobioreactor using acrylic sheet b) Fixing of LED lights on photobioreactor for illumination c) Algal cultures efficiently growing in bottles fixed with silicone bottle brush agitator system

Figure 2. Closer view of Agitator made using silicone bottle brush

ACKNOWLEDGEMENTS
The authors are thankful to Jai Hind College for providing Central Instrumentation Facility Laboratory of Jai Hind College for carrying out practical work. Authors are also thankful to Cyanohealth Research Centre situated at Borivali, Mumbai for facilities provided for carrying out this research work.

REFERENCES

Balaji S, Kalaivani T, Rajasekaran C, Shalini M, Vinodhini S, Priyadharshini SS & Vidya AG. 2015. Removal of heavy metals from tannery effluents of Ambur industrial area, Tamilnadu by Arthrospira (Spirulina) platensis. *Environmental Monitoring and Assessment*, **187**(6):325. https://doi.org/10.1007/s10661-015-4440-7

CZ. 1966. Contribution a l'etude d'une Cyanophycee. Influence de Divers Facteurs Physiques et Chimiques sur la croissance et la photosynthese de Spirulina mixima. *Thesis. University of Paris, France.* https://cir.nii.ac.jp/crid/1571698599087861632

Cao G & Prior RL. 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry*, **44**(6 Pt 1):1309–1315.

Coronado-Reyes JA, Salazar-Torres JA, Juárez-Campos B & González-Hernández JC. 2020. *Chlorella vulgaris,* a microalgae important to be used in Biotechnology: A review. *Food Science and Technology*, 42, e37320. https://doi.org/10.1590/fst.37320

Fagiri YMA, Salleh A, & El-Nagerabi SAF. 2013. Impact of physico-chemical parameters on the physiological growth of Arthrospira (Spirulina platensis) exogenous strain UTEXLB2340. *African Journal of Biotechnology*, **12**(35), Article 35. https://doi.org/10.5897/AJB2013.12234

Gong M & Bassi A. 2016. Carotenoids from microalgae: A review of recent developments. *Biotechnology Advances*, **34**(8):1396–1412. https://doi.org/10.1016/j.biotechadv.2016.10.005

Halliwell B & Gutteridge JM. 1990. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods in Enzymology*, **186**:1–85. https://doi.org/10.1016/0076-6879(90)86093-b

Hu Q, Pan B, Xu, J, Sheng J & Shi Y. 2007. Effects of supercritical carbon dioxide extraction conditions on yields and antioxidant activity of Chlorella pyrenoidosa extracts. *Journal of Food Engineering*, **80**(4): 997–1001. https://doi.org/10.1016/j.jfoodeng.2006.06.026

Ismaiel MMS, El-Ayouty YM, & Piercey-Normore M. 2016. Role of pH on antioxidants production by *Spirulina* (*Arthrospira*) *platensis*. *Brazilian Journal of Microbiology*, **47:**298–304. https://doi.org/10.1016/j.bjm.2016.01.003

Mubarak M, Shaija A & Suchithra TV. 2020. Evaluation of ferric chloride and electroflocculation of Chlorella pyrenoidosa and reuse of the culture medium for subsequent cultures. *Journal of Environmental Chemical Engineering*, **8**(1):103612. https://doi.org/10.1016/j.jece.2019.103612

Park WS, Kim HJ, Li M, Lim DH, Kim J, Kwak SS, Kang CM, Ferruzzi MG & Ahn MJ. 2018. Two Classes of Pigments, Carotenoids and C-Phycocyanin, in Spirulina Powder and Their Antioxidant Activities. *Molecules*, **23**(8), Article 8. https://doi.org/10.3390/molecules23082065

Perera RMTD, Herath KHINM, Sanjeewa KKA & Jayawardena TU. 2023. Recent Reports on Bioactive Compounds from Marine Cyanobacteria in Relation to Human Health Applications. *Life*, **13**(6), Article 6. https://doi.org/10.3390/life13061411

Ramlee A, Rasdi W & Abd ME. 2021. Microalgae and the factors involved in successful propagation for mass production. *Journal of Sustainability Science and Management*, **16**(4):21–42.

Rana, M. S., Bhushan, S., Sudhakar, D. R., & Prajapati, S. K. 2020. Effect of iron oxide nanoparticles on growth and biofuel potential of Chlorella spp. *Algal Research*, *49*, 101942. https://doi.org/10.1016/j.algal.2020.101942

Rao AV & Rao LG. 2007. Carotenoids and human health. *Pharmacological Research*, *55*(3), 207–216. https://doi.org/10.1016/j.phrs.2007.01.012

Ren, Y., Deng, J., Huang, J., Wu, Z., Yi, L., Bi, Y., & Chen, F. (2021). Using green alga Haematococcus pluvialis for astaxanthin and lipid co-production: Advances and outlook. *Bioresource Technology*, *340*, 125736. https://doi.org/10.1016/j.biortech.2021.125736

Sharma R, Singh GP & Sharma VK. 2012. Effects of culture conditions on growth and biochemical profile of Chlorella vulgaris. *Journal of Plant Pathology and Microbiology*, *3*(5), 1–6.

Soni RA, Sudhakar K & Rana RS. 2017. Spirulina – From growth to nutritional product: A review. *Trends in Food Science & Technology*, *69*, 157–171. https://doi.org/10.1016/j.tifs.2017.09.010

Soni RA, Sudhakar K & Rana RS. 2019. Comparative study on the growth performance of Spirulina platensis on modifying culture media. *Energy Reports*, *5*, 327–336. https://doi.org/10.1016/j.egyr.2019.02.009

Stahl W & Sies H. 2005. Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1740*(2), 101–107. https://doi.org/10.1016/j.bbadis.2004.12.006

Tapiero H, Townsend DM & Tew KD. 2004. The role of carotenoids in the prevention of human pathologies. *Biomedicine & Pharmacotherapy*, *58*(2), 100–110. https://doi.org/10.1016/j.biopha.2003.12.006