EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 1
302-304
Year: 2019

SUITABLE ASSESSMENT OF THE EXTRACTION AND QUANTITY OF CHLOROPHYLL OF ECO-FRIENDLY CYANOBACTERIA

Anil Kumar[3], Asha D. Lazarus[2], B. D. Nagale*[1], S. R. Gayakwad[3], S. Rane[3], R. Hanote[3], K. Khasdeo[3]

[1]J. H. Govt. P. G. College Betul MP.
[2]M. L. B. Govt. Girls P. G. College Bhopal MP.
[3]Vivekanand Science College (VVM) Betul MP.

***Corresponding Author: B. D. Nagale**
J. H. Govt. P. G. College Betul MP.

Article Received on 12/11/2018 **Article Revised on 04/12/2018** **Article Accepted on 25/12/2018**

ABSTRACT
This study of chlorophylls amount presented in the cyanobacteria cells was influenced by exposure to light and dark reaction at several times intervals. Simply methods were applied to chlorophylls extraction from cyanobacterial cells, while cell wall was raptured through mechanical approaches in the presence of hexanol organic solvent. Further, estimated analysis of quantities of chlorophylls by the UV-Vis spectrophotometer were obtained results such as 14 hours light and 10 hours dark reaction at 25 °C which have contains a good percentage of chlorophylls, while 12 hours is equivalent time to the light and dark reaction occurs at 20 °C, got a fair percentage of chlorophylls.

KEYWORDS: Chlorophylls, Cyanobacteria, Hexanol, UV-Vis Spectrophotometer.

INTRODUCTION
The present research was focused of effect of light and dark times on the chlorophylls amount presented in cyanobacterial biomass from different atmospheric condition such as temperature of aquatic system. The chlorophylls (green color pigments) contented in the cyanobacteria is a specific feature of each species. In the ability of photosynthetic cyanobacteria "blue green algae" is usually found in free-floating on surface water.[1] It is evaluated as the parameters of indirect measurement of cell growth with the investigation of water trophic level. Several aquatic scientists are monitoring the water resources in which Phytoplankton has been found to improve the perceptions on nutrition status in the aqueous system, the biomass of algae bloom prospectus[2]. Several types of cyanobacteria are floating on the surface of water for production in different sizes and sizes. The green colour is visible in the cyanobacteria, which is highly concentrated in chlorophyll pigment within the cells, other types of pigments such as phycobiliproteins and carotenoids are formed within their cells.[2] Cyanobacteria were the biological material used for the study of chlorophyll is the richest pigment in this species. Therefore, cyanobacteria are commonly known to as the primary producer of aquatic food web, which is accompanied by the biomass of the accessories pigments phycocyanin and phycoerythrin.[3] Chlorophyll provides a dynamic function by absorbing light energy in natural form from sun during photosynthesis to derivate a reaction mechanism in which dissolved CO_2 in H_2O are produce $C_6H_{12}O_6$ (sugar molecules) as know nutrient supplements and originated O_2 gas are utilised by aquatic living organism in respiration.[4-6] Comparison of different photoreaction periods effects to measure chlorophylls is used as the time needed for the quantity of pigment identified as selection criteria and analysis. In additional to the mechanical approach were applied on cyanobacterial cells with using hexanol organic solvent capable to raptured the cell wall to the extraction of chlorophylls. Many methods in literature can be based on a good tool to evaluate and analysis of chlorophylls by UV-Vis. spectrophotometer. Evaluation of different photoreaction periods effects to quantification measure of chlorophylls is used identified as selection criteria and analysis.

MATERIALS AND METHODS
The cyanobacteria cultured in BG11 media were maintained in the laboratory culture room. The cultures were kept for light and dark period at different times such as L12/D12, L14/D10 and L16/D8 (where as L=Light and D= Dark) at such varied temperatures 20°C, 25°C and 30°C for 15 days in BOD shaker was used for maintained of temperature and atmosphere as well as required for growth of cyanobacteria. After 15 days old, the volume of 5 ml cultured samples were taken for the extraction of total chlorophyll (green pigment) in the centrifuge tubes, the cultures were centrifuged at 5000 rpm for 5 minutes and the pellet of the algal biomass was

collected and discarded of the supernatant and pellet were re-suspended in 5 ml of distilled water to remove culture media salts and again to centrifugation according to above protocol exercise. The washing process was repeated 3-4 times to insure removed impurity from biomass and it was transferred in mortal and suspended in 2 ml of hexanol organic solvent with help of vortex mixing for 1 minute after that added some amount of sea beads and was grinded at homogenate form and final volume 5 ml of solution was maintained with hexanol, used in chlorophylls extraction. Then it was again centrifuged at 5000 rpm for 10 minutes and supernatant were collected in the fresh tubes for further analysis. The supernatant absorbance read at the corresponding wavelength by UV-Vis. Spectrophotometry was used empirical estimation of total chlorophyll assessment.

RESULTS AND DISCUSSIONS

According to obtained the results from nine samples of cyanobacteria *in vitro* cultured in laboratory were empirical protocol of this projects such as cultured, chlorophyll isolation and quantification chlorophylls, monitoring of the effect of photosynthetic mechanisms such as different temperatures with different light and dark reaction exposed of the aspect of cyanobacteria to produce the quantities of chlorophylls was determined by the UV-Vis spectrophotometer. Where the samples were projected in S1 to S9 replicates of cyanobacteria and applied three temperatures such as 20 °C, 25 °C and 30 °C with different light and dark reaction times such as 12/12, 14/10 and 16/8 for 15days. The details showed in table No1.

The cyanobacteria cultured setup first for three replicates at different temperatures and constant of light/dark reaction duration equal 12 hours was observed after 15 days in which samples S1 at 20 °C colour appeared yellow, S2 at 25 °C colour appeared light green and S3 at 30 °C colour appeared light yellow. It was varied for the improper development of chlorophyll pigment in thallus of cyanobacteria, because different temperature may be possible to change the visual varied status of cyanobacteria. The details showed in figure No1.

The cyanobacteria cultured setup second for three replicates at different temperatures and constant of light/dark reaction duration 14 hours for light and 10 hours for dark was observed after 15 days in which samples S4 at 20 °C colour appeared light yellow, S5 at 25 °C colour appeared dark green and S6 at 30 °C colour appeared green. It was varied for the good development of chlorophyll pigment in thallus of cyanobacteria. The details showed in figure No 2.

The cyanobacteria cultured setup second for three replicates at different temperatures and constant of light/dark reaction duration 16 hours for light and 8 hours for dark was observed after 15 days in which samples S7 at 20 °C colour appeared light yellow, S8 at 25 °C colour appeared green and S9 at 30 °C colour appeared light green. It was varied for the average development of chlorophyll pigment in thallus of cyanobacteria. The details showed in figure No 3.

The calculation of percentages of estimated result found between the samples S1 to S9 in randomly form in increasing order according quality of chlorophylls green pigments such as S1<S7<S3<S4<S9<S2<S6<S8<S5. The details showed in figure No 4.

Table No. 1: showed data of the samples S1 to S9 treated of light and dark reaction times exposed for 15 days and know concentration chlorophyll by UV-Vis spectroscopy at 450 nm.

Samples	Cyanobacteria cultured for 15 days		Temperature in °C	Chlorophyll OD at λ, 450 nm
	Light in hrs	Dark in hrs		
S1	12	12	20	2.252
S2	12	12	25	2.474
S3	12	12	30	2.359
S4	14	10	20	2.361
S5	14	10	25	2.684
S6	14	10	30	2.518
S7	16	8	20	2.351
S8	16	8	25	2.575
S9	16	8	30	2.438

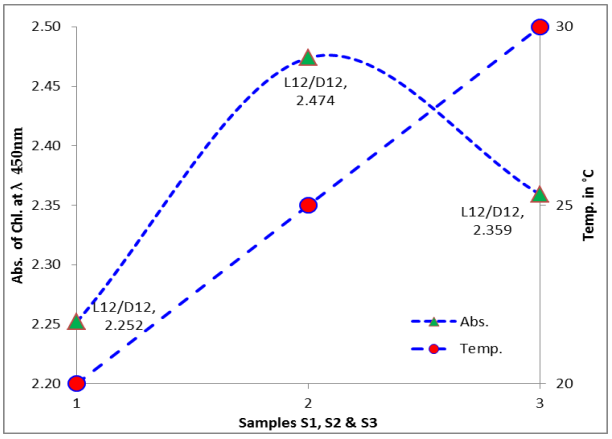

Figure No. 1: showed spectroscopy absorption graph of chlorophylls the samples as S1, S2, and S3 treated equals times12 hrs of light and dark reaction at different temperatures period for 15 days.

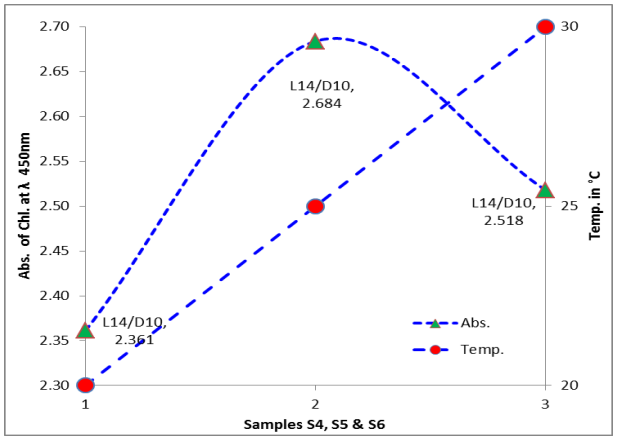

Figure No. 2: showed spectroscopy absorption graph of chlorophylls the samples as S4, S5, and S6 treated times 14 hrs light and 10 hrs dark reaction at different temperatures period for 15 days.

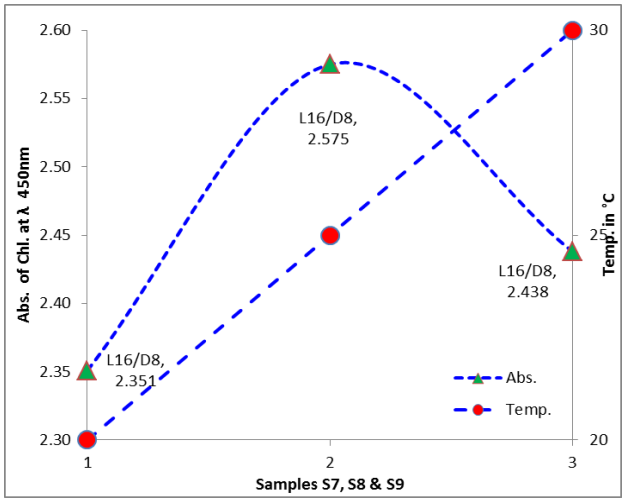

Figure No. 3: showed spectroscopy absorption graph of chlorophylls the samples as S7, S8, and S9 treated times 16 hrs light and 8 hrs dark reaction at different temperatures period for 15 days.

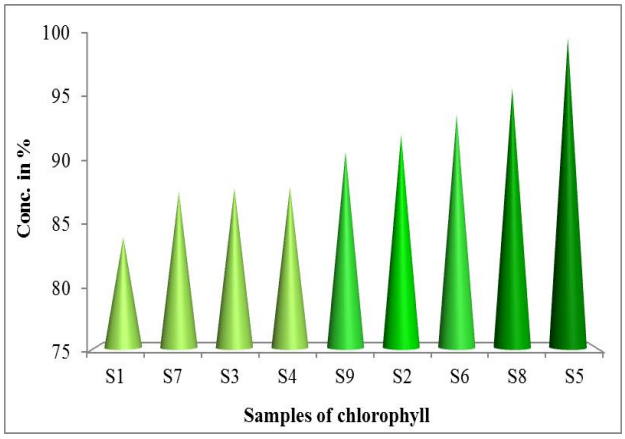

Figure No4: showed diagram of the samples S1 to S9 percentages quantities of chlorophylls in randomly increasing order.

CONCLUSIONS

Find out the water borne microorganisms such as algae cyanobacteria culture was exposed of sunlight at difference time intervals for estimation of optimum quantities of chlorophyll. Whereas a healthy species is the source of better yield. Various temperatures and light and dark periods were affected by cyanobacteria growth rate and chlorophyll concentrations.

REFERENCES

1. J G Day, T Proschold, T Friedl, M Lorenz, P C Silva. Conservation of microalgal type material: Approaches needed for 21st century science. Taxon, 2010; 59: 3-6.
2. D J Gerla, W M Mooij, J Huisman. Photoinhibition and the assembly of light-limited phytoplankton communities. Oikos, 2011; 120: 359-368.
3. C L Colyer, C S Kinkade, P J Viskari, J P Landers. Analysis of cyanobacterial pigments and proteins by electrophoretic and chromatographic methods. Anal Bioanal Chem, 2005; 382: 559-569.
4. A K Singh, L A Sherman. Iron-independent dynamics of IsiA production during the transition to stationary phase in the cyanobacterium Synechocystis sp. PCC 6803. FEMS Microbiol. Lett. 2006; 256: 159-164.
5. H Nagase, K Yoshihara, K Eguchi, Y Okamoto, S Murasaki, R Yamashita, K Hirata, K Miyamoto. Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae. Biochem. Eng. J., 2001; 7: 241-246.
6. A P Allen, J F Gillooly, J H Brown. Linking the global carbon cycle to individual metabolism function. Ecol., 2005; 19: 202-213.