



Estimation of Total Chlorophyll and Total Carotenoid Contents for Strains of Anabaena and Gloeocapsa Through Spectrophotometer

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ABSTRACT

During experimental period growth for two micro algal strains were optimized through different media. Total Chlorophyll and Carotenoid contents from *Anabaena sp.* and *Gloeocapsa sp.* were estimated using spectrophotometer. The total chlorophyll content has been recorded as maximum 7.080 $\mu\text{g/ml}$ from *Gloeocapsa sp.* in Bold Basal medium (pH 7.0) and 20.939 $\mu\text{g/ml}$ from *Anabaena sp.* in BG-11 medium (pH 7.1). *Gloeocapsa sp.* showed the highest carotenoid content at 1.993 $\mu\text{g/ml}$ in Pringsheim's medium (pH 7.1) and the same result for *Anabaena sp.* was at 5.653 $\mu\text{g/ml}$ in BG-11 medium.

KEYWORDS : Microalgae, Chlorophyll, Carotenoid, Spectrophotometer, Culture media

Introduction:

The term Chlorophyll derived from two Greek words "Chloros" which means Green and "Phyllon" a term for Leaf. This term is used for several closely related green pigments found chiefly in chloroplast of algae and green plants. It is an important bio-molecule which play crucial role in photosynthesis by absorbing energy from light in the form of photons. In photosynthetic machinery chlorophyll is used to pump H^+ ions across the thylakoid membrane to produce energy rich ATP molecules. At molecular level it is a cyclic tetrapyrrole, like the heme group of globins and cytochromes which is derived biosynthetically from protoporphyrin IX. The central metal ion of chlorophyll is Mg^{2+} . Studies reveal that about 50% of world photosynthesis is carried out by algae. Recent research suggests that chlorophyll molecules also play a vital role in promoting good health. For instance, it helps in hunger control, healings, DNA protection from fried foods, cancer therapy, swellings, and iron supplements.

Carotenoids are accessory pigments found in photosynthetic organisms. They are derivatives of tetraterpenes and categorized into two major classes, namely xanthophylls and carotenes. They play two extremely important roles in algae and green plants. Firstly they absorb light energy during photosynthesis and secondly they protect chlorophyll molecule from photo damage. According to recent studies carotenoids play important role to human health as antioxidants, combating tumor development, increasing male fertility, supporting cardiovascular and eye health and so on.

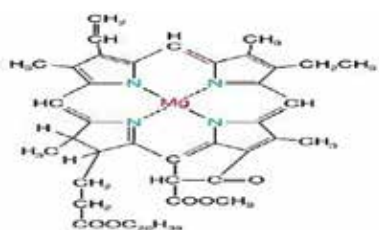


Fig.1. Structure of Chlorophyll

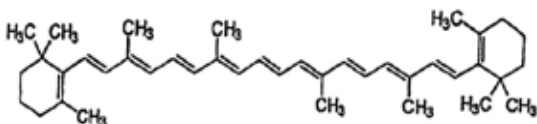


Fig.2. Structure of Carotenoid

Materials and Methodologies
Preparation of culture media

Algae media refers to the solution or culture in which algae grow, and there are two major types of algae media, enrichment and artificial media. An enrichment medium is generally made by adding soil extracts to distilled or natural water or by simply adding chemical nutrients to seawater or lake/dam water. The artificial medium uses "pure" water and "pure" chemicals and doesn't include additions of soil ex-

tracts or natural lake or sea water. This artificial medium is mostly used under laboratory conditions to exacting standards, although unknown impurities can still be present in even the most carefully prepared artificial medium.

The most important aspects in algae growing conditions are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. Concentrations of algae in media are generally much higher than those found in nature, so the media or culture must be enriched with nutrients to boost the water in order to support higher cell densities.

In this study the following culture media were used**1. BG-11 (pH 7.1)**

Sl No	Ingredients	Gm/L
1	NaNO_3	1.5
2	K_2HPO_4	0.0314
3	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.036
4	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.0367
5	Na_2CO_3	0.020
6	$\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$	0.001
7	Citric acid. H_2O	0.0056
8	Ferric ammonium citrate	0.006

2. Bolds Basal Medium (pH 7.0)

Sl No	Ingredients	Gm/L
1	NaNO_3	25
2	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	2.5
3	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	7.5
4	K_2HPO_4	7.5
5	KH_2PO_4	17.5
6	NaCl	2.5
7	EDTA	50.0
8	KOH	31.0
9	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	4.98
10	H_2BO_3	11.42
11	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.82
12	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.44
13	MoO_3	0.71
14	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1.57
15	$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.49
16	H_2SO_4	1.0 ml

3. Pringsheim's Medium (pH 7.1)

Sl No	Ingredients	Gm/L
1	KNO ₃	0.200
2	MgSO ₄	0.010
3	(NH ₄) ₂ HPO ₄	0.020
4	CaCl ₂	0.005
5	FeCl ₃	0.0005

*Vitamins

Thiamine- 1g

Biotin- 1g

Nicotinic acid- 1g

Cyanocobalamine- 1ml

Total Chlorophyll and Carotenoid extraction and measurement through Spectrophotometer

The following steps are followed for the efficient measurement of chlorophyll and carotenoids.

- A volume of 2 mL culture sample was withdrawn.
- Cells were centrifuged at 3000 rpm for 10 min.
- The supernatant was removed and cells were then re-suspended in 2 mL of distilled water to remove any salts that could have been retained with biomass, and submitted again to centrifugation. This washing process was repeated twice
- Washed cells were suspended in 2 mL of 99.8% methanol with strong vortex mixing for 15 s.
- Optical density was measured for each sample with the help of Spectrophotometer.

Equations

$\mu\text{gChlorophyll}/\text{mLmedium} = 15.65 A_{666}$

$\mu\text{gtotal carotenoid}/\text{mLmedium} =$

$$\frac{1000A_{470} - 44.76A_{666}}{221}$$

221

Result

Media	Strain	Chlorophyll($\mu\text{g}/\text{ml}$)	Carotenoid ($\mu\text{g}/\text{ml}$)
BG-11	<i>Gloeocapsa</i> sp.	6.90	1.706
BG-11	<i>Anabaena</i> sp.	20.939	5.653
Bold Basal	<i>Gloeocapsa</i> sp.	7.080	1.917
Bold Basal	<i>Anabaena</i> sp.	14.590	4.402
Pringsheims	<i>Gloeocapsa</i> sp.	6.00	1.993
Pringsheims	<i>Anabaena</i> sp.	12.484	3.628

Discussion

The chlorophyll and carotenoid contents of algae would play a great importance to human health. Now a day due to some factors such as overcrowding, radiations and pollution of air, water or sound the quality of life is affected by several disorders. The supplement of these molecules through food is found to be beneficial to combating such disorders related to human health. To grow and culture algal strains for the production of these vital bio-molecules proper culture media and methods of growth optimization should be implemented.

Acknowledgement

This study was supported by Department of Botany, Dhing College, Dhing-782123, Nagaon, Assam, India.

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