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COMPARISON OF DIFFERENT MEDIA FORMULATIONS ON GROWTH, MORPHOLOGY AND CHLOROPHYLL CONTENT OF GREEN ALGA, *CHLORELLA VULGARIS*

REKHA SHARMA[1], GAJENDRA PAL SINGH[2] AND VIJENDRA K. SHARMA*[1]

[1]Department of Botany, MSJ Government PG College, Bharatpur-321 001, Rajasthan (India)
[2]Department of Botany, University of Rajasthan, Jaipur-302 055, Rajasthan (India)

VIJENDRA K. SHARMA

PG Department of Botany, MSJ Government PG College, Bharatpur-321 001, Rajasthan (India)
Email: sharmavijendrak@yahoo.co.in

ABSTRACT

Chlorella vulgaris is a green, spherical, single celled fresh water microalga belongs to the phylum Chlorophyta. As per the study conducted so far it is found that green algae are the highest source of chlorophyll in the plant world and particularly, *Chlorella* one of the members of green algae is the richest source of chlorophyll which is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry. *Chlorella* has been produced commercially in several countries for its use as neutraceutical food and medicinal purpose due to its valuable contents particularly pigments and proteins. The aim of this work is to evaluate the effect of five different defined inorganic medium such as Juller's medium, Bold's basal medium, modified Chu-10 medium, N-8 medium, and Kuhl's medium, on the growth, morphology and pigment content of *C. vulgaris*. The best growth was obtained in modified Chu-10 medium as compared to other medium.

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KEY WORDS

Chlorophyll content, *Chlorella vulgaris*, Growth medium, Morphology

INTRODUCTION

In the early 1950's, the increase in the world's population and predictions of an insufficient protein supply led to a search for new alternative and unconventional protein sources. Algal biomass appeared at that time as a good candidate for this purpose[1, 2]. Interest in applied algal culture continued with studies of the use of algae as photosynthetic gas exchangers for space travel and *Chlorella* as an ideal food for long-term space travel and colonization[3]. *Chlorella* is the most cultivated eukaryotic green micro alga as it is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry. It contains proteins, carotenoids, lipids, immunostimulator compounds, polysaccharides, vitamins, antioxidants and minerals[4]. Other health promoting effects of *Chlorella* are the lowering of cholesterol, preventive action against atherosclerosis or antitumor action. Extracts of *C. vulgaris* are considered to have antimicrobial effects[5].

To produce high quality biomass, attention must be paid to culture status. Large-scale production of *Chlorella* biomass depends on many factors, the most important of which are nutrient availability, temperature and light. These factors influence the growth of *Chlorella* and the composition of the biomass produced by causing changes in metabolism. Number of media compositions for the cultivation of microalgae has been proposed. These media compositions were based on the analysis of the natural habitats where the algae grows[6]. The growth of *Chlorella* at various concentrations of macro-nutrients and micro-nutrients has been studied[7] and some media formulations have been made which were based on detailed study of nutrient requirements of algae[8].

The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn, and Zn[9] and these elements are added in the form of salts, a list of which is found in Kaplan and co-workers published work[10]. There have been extensive studies on the growth of *Chlorella* in culture media with different concentration of different carbon sources[11, 12]. Variation in the elemental composition of *Chlorella* under different conditions and different stages of growth has been reported[9, 13]. The objective of the present study is to evaluate the influence of different media on the growth and pigment content of *Chlorella vulgaris* to find out best defined inorganic media for cultivation of *C. vulgaris.*

MATERIALS AND METHODS

The experimental organism *Chlorella vulgaris* was isolated from Mawtha, a fresh water pond, pH 7.3, near Amber Fort in Jaipur, Rajasthan, cultured on Juller's medium[14] and maintained on the same medium by regular subculturing in every two weeks. Experiments to evaluate the effect of different media on *C. vulgaris* were carried out in the departmental laboratory.

In order to find out the best culture medium, cultures were subjected to five different media of different chemical compositions and pH, as mentioned below:-

1. Juller's medium[14]
2. Bold's basal medium[15]
3. Modified Chu-10 medium[16]
4. N-8 medium[17]
5. Kuhl's medium[18]

Three test tube sets of each medium containing 10 ml of medium and 2 ml of freshly growing cultures were subjected to different

medium and there growth were followed through optical density (OD) and cell count (CC). Simultaneously, five conical flasks containing 250 ml of each medium and 50 ml *C. vulgaris* were subjected for pigment estimation. All medium in the flask and test tubes were sterilized in autoclave at 121°C for 20 min. before inoculation. The cultures were incubated at 25°C in a thermo-statically controlled room, with a 12:12 h light dark regime.

Observations were carried out over a period of five weeks after initial readings. Growth was followed through optical density, cell count and pigment content. Optical density was recorded by using colorimeter at 670nm and cell count examination was performed using haemocytometer (Neubauer improved). UV visible spectrophotometer was used for pigment estimation in different cultures of *C. vulgaris.* The chlorophyll-a and chlorophyll-b content of the samples were estimated by Parson and Strickland method[19]. Cultures were shaken gently thrice a day to avoid clumping and accelerate the growth process. Experiment for each medium was carried out in triplicates.

RESULTS AND OBSERVATIONS

Estimation of growth (OD and CC) and chlorolphyll content of *C. vulgaris* in different media shows different growth pattern and chlorophyll content, among the all five media, modified Chu-10 medium shows maximum growth followed by Bold's basal medium, Juller's medium, N-8 medium and minimum growth was observed in Kuhl's medium.

Optical density and cell count clearly indicated that the best growth of *C. vulgaris* was obtained in modified Chu-10 medium as compared to that in other media. OD and CC had increased by 4.4 times and 4.2 times of the initial record respectively, after a period of five weeks (Table -1 and Table -2). Bold's basal medium was next to modified Chu -10 solution in promoting the growth of *C. vulgaris*. Growth was increased about 3.2 times in terms of OD and 2.9 times in terms of CC. In Juller's medium OD and CC were increased 2.7 and 2.1 times, respectively, of the initial record. In N-8 medium OD was increased 2.5 times and CC was increased 2.0 times the initial record. Khul's medium proved to be insufficient in supporting the growth of *C. vulgaris* as has been observed through OD as well as CC (Table 1 and Table 2), which increased up to 1.4 and 1.9 times the initial record, respectively.

Table 1
Effect of different media on growth (OD) of Chlorella vulgaris

Week	Juller's	BBM	Chu-10	N-8	Kuhl's
Initial	0.10 ±.012	0.10±.012	0.10±.012	0.10±.012	0.10±.012
1st	0.13±.020	0.14±.016	0.14±.030	0.12±.008	0.13±.016
2nd	0.17±.016	0.21±.012	0.24±.029	0.14±.020	0.15±.016
3rd	0.21±.012	0.25±.016	0.27±.016	0.24±.026	0.18±.012
4th	0.23±.024	0.28±.008	0.37±.012	0.23±.028	0.20±.024
5th	0.27±.024	0.32±.020	0.44±.021	0.25±.040	0.19±.016

Each value is Mean±SD (n=3), 0ptical density (OD)

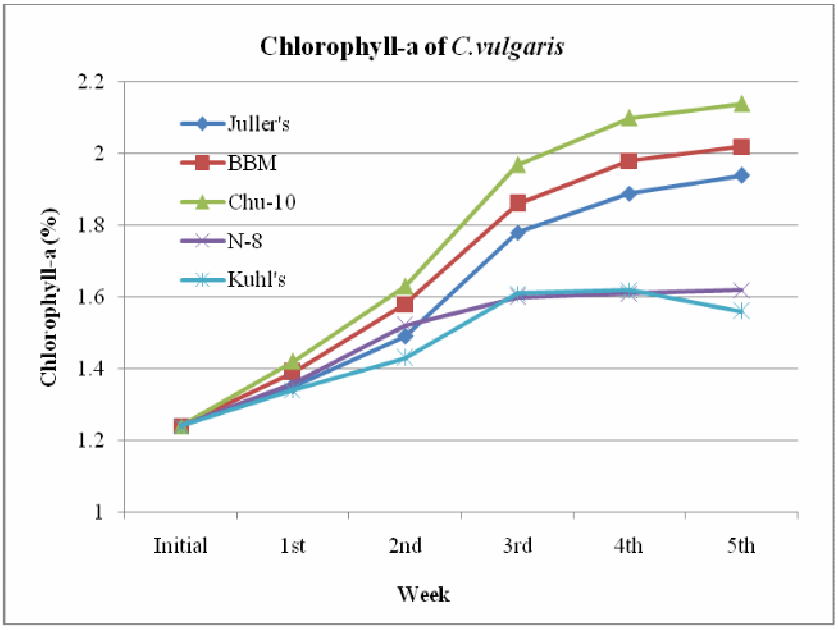
Table 2
Effect of different media on growth (CC) of Chlorella vulgaris

Week	Juller's	BBM	Chu-10	N-8	Kuhl's
Initial	100×10⁴	104×10⁴	96×10⁴	96×10⁴	98×10⁴
1st	110×10⁴	140×10⁴	120×10⁴	145×10⁴	105×10⁴
2nd	150×10⁴	204×10⁴	208×10⁴	175×10⁴	167×10⁴
3rd	206×10⁴	245×10⁴	278×10⁴	200×10⁴	189×10⁴
4th	217×10⁴	279×10⁴	340×10⁴	221×10⁴	212×10⁴
5th	210×10⁴	305×10⁴	408×10⁴	198×10⁴	187×10⁴

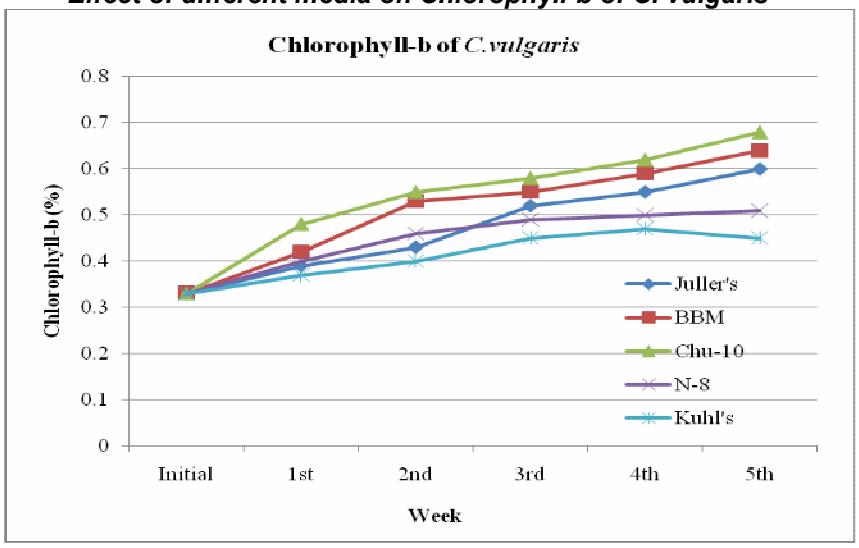
Each Value is the average of three replicates, Cell count (CC)

The pigment content of the algae also correlates with the growth of *C. vulgaris.* Maximum Chl-a and Chl-b content were found in cultures of modified Chu-10 medium i.e. 2.14% and 0.68%, respectively, after a period of five weeks, followed by 2.02% and 0.64% in Bold's basal medium, 1.94% and 0.60% in Juller's medium, 1.62% and 0.51% in N-8 medium and minimum Chl-a and Chl-b content were found in Kuhl's medium i.e. 1.56% and 0.45%, respectively (Graph 1 and Graph 2).

Graph 1
Effect of different media on Chlorophyll-a of C. vulgaris



Graph 2
Effect of different media on Chlorophyll-b of C. vulgaris



The growth rate of the algae seemed to be directly associated with morphological configurations. The normal morphological configuration was observed in modified Chu-10 medium. Most of the cells were healthy, bright green and having intact chloroplast up to four weeks. Four week onwards certain cells were noted to be unhealthy with broken chloroplasts. Bold's basal medium was second best medium for promoting the growth of *C. vulgaris*. In this culture, healthy appearance of the cells was maintained up to three weeks with the exception of the few cells. Subsequent observations revealed unhealthy and fragmentary chloroplast. Juller's medium was found third best medium in terms of growth. In this medium cells were having green, intact chloroplasts, but some unhealthy cells were also seen in the third week. From third week onward, cultures became yellowish green with broken and fragmented chloroplasts. N-8 medium was found next to Juller's medium for measured the growth of *C. vulgaris*. The cells were normal green with intact chloroplasts up to second week. After second week onwards cultures appeared unhealthy. The chloroplasts showed contraction, fragmentation and granulation. The color also finally turned yellowish-green. A very poor morphological configuration was showed in Kuhl's medium, where cells were faint green with granular fragmented and slightly shrunken chloroplasts from the periphery. Some cells with vacuolated chloroplasts were also observed after second week onwards.

DISCUSSION

Five inorganic defined medium varying in their chemical composition and pH, in which, modified Chu-10 medium proposed best for growth (OD and CC), pigment complex and morphology. Similar observations were also reported by many scientists i.e. nutritional studies with variations in the amounts of essential elements in the solution may show that modifications of Chu No. 10 will result in a faster rate and greater amount of growth of many of the algae[20].

The growth of *C. vulgaris* in different culture media was primarily followed by optical density, counting algal cells and chlorophyll estimation. Chlorophyll measurement did not directly coincide with direct cell counts and the discrepancy in chlorophyll concentration is likely due to the variability of levels within

individual cells, and not as a result of changes in the overall biomass[12].

K_2HPO_4 was the source of phosphate in modified Chu-10 medium, it may be responsible for the rapid growth of the alga under experiment, as has earlier been reported in *Selenastrum* and this phenomenon assigned to the enhanced dark reaction[21], while other nutrients were limiting. It is often assumed that the limiting nutrients are nitrogen and phosphorus (elements that comprise higher percentages in the cellular composition).

Nitrogen being important constituent of the cell protein was needed for algal growth, either in combined or in molecular form. In modified Chu-10 medium $Ca(NO_3)_2$ $4H_2O$ at higher pH led to precipitate formation in the medium but lower pH of the medium prevent the precipitation. The gradual rise in pH in cultures using nitrate only, though troublesome because of reduction in the solubility of phosphate salts at the higher pH[22]. As nitrogen deficiency develops the amount of chlorophyll in the cells decreases faster than the nitrogen content in *C. vulgaris* cultures[17].

$MgSO_4$ was the source of magnesium in modified Chu-10 medium; it is permitted the maximum growth of the present alga and magnesium deficiency interrupted cell division in *Chlorella* which results in abnormally large cell formation[23]. Increase in magnesium alone in the medium resulted in higher cell number, although increase in nitrogen alone did not make much difference that means cells need magnesium to synthesize chlorophyll[17]. The process of multiplication requires a larger concentration of magnesium in the medium than does the production of cell material[24].

Iron uptake is strictly required for phytoplankton development, because in the absence of iron, retardation of growth, reduction of photosynthetic activity and chlorophyll content is observed[25]. Ferric citrate and citric acid combination was the source of iron in modified Chu-10 medium by substituting an organic source of iron, ferric citrate, for the ferric chloride. This improvement is due to increased iron availability[26]and an equal amount of citric acid with the ferric citrate stabilized the concentration of reactive iron in the nutrient solution.

The growth of microalgae not only depends on the temperature, light and nutrient availability, but also has a direct impact on the available carbon in the culture medium. In modified Chu-10 medium carbon source was Na_2CO_3, higher chlorophyll content in this medium showed that Na_2CO_3 support the growth of *C. vulgaris.* Carbon to chlorophyll ratio is a sensitive indicator of the physiological state of microalgae[11]. Sodium carbonate and sodium silicate provides alkaline buffering in the medium.

Trace metal mixture and Na_2EDTA were included in the composition of modified Chu-10 medium. The research establishing requirements of various species of algae for manganese, zinc, calcium, boron, and possibly copper but trace metals can act as a nutrient but at an elevated concentration they can interact with proteins and can change the structure and enzymatic activities within the cell of an aquatic organism and can display its toxic effects at the whole organism level[27]. EDTA increases the density to which a population can be carried and that without it some factor, presumably availability of microelements, soon becomes severely limiting. Consideration of the function of a chelating agent shows that it will be a very great aid in mass culture work. It is a serious problem to provide adequate amounts of the various microelements, and to maintain their availability, without exceeding the limits of toxicity. The easily reversible chelate complex provides a buffer system that will maintain ionic

concentrations at desirable levels throughout the life of a culture.

It has mentioned above that the water body has pH 7.3 from where the algae have been isolated. The pH of modified Chu-10 medium was maintained between 7.2 to 7.4, thus this medium favours the growth of algae. The pigment content of *C. ellipsoidea* was highest at pH 4.0, 6.0 and 7.5[28] and some scientists maintained unialgal and axenic cultures of *C. vulgaris* in modified Chu-10 medium in their research work but they had not mentioned the impact of this medium on growth and morphology of *C. vulgaris*[29-31]. The green alga *Botryococcus protuberans* has shown enhanced growth rate in the modified Chu-10 medium[32].

The performance of different species in different media may also be conditioned by the previous history of the cells[22]. In previous studies it has been shown that modified Chu-10 medium support the growth of blue green algae. However, in our experiments, the modified Chu-10 medium supports the growth of green alga, *Chlorella vulgaris*.

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