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Optimization of various culture media for mass production of selected microalgae

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Abstract
Microalgae perform as a source of biologically active compounds. However, its mass production is essential to isolate the adequate quantities of the bioactive components. Growth and multiplication of three microalgae, *Chlorella ellipsoidea*, *Chlorella vulgaris* and *Coelastrum* sp belonging to class Chlorophyceae was studied in the laboratory, using four different culture media viz., Bold's Basal Medium, CHU10, Walne's and 3N BBM+Vitamin medium. The experiment was conducted for a period of 30 days under controlled conditions of temperature (25°C) and light (1 k lux). The three different microalgae shows varied growth pattern in different culture media. The alga *Chlorella ellipsoidea* attained maximum cell density (5.8×10^6 cells/ ml) in 3N BBM +Vitamin medium within 27 days. *Chlorella vulgaris* reached maximum cell density (26.7×10^6 cells /ml) in BBM within 18 days and the maximum growth was observed in Chu 10 medium for *Coelastrum* sp with a cell density of 8.72×10^6 cells/ml, within 18 days. The optimized medium of each microalga can be used for their large-scale cultivation, biomass accumulation and isolation of bioactive components.

Keywords: Microalgae, *Chlorella ellipsoidea*, *Chlorella vulgaris*, *Coelastrum* sp

1. Introduction
Microalgae have received a lot of attention in the scientific community and biotechnology industry due to the fact that many microalgae strains are very good sources of various biologically active compounds. The use of micro algal bio-mass proved to be advantageous in agriculture that they are highly productive on arable and non-arable land. They can also adapt to varying environmental conditions by producing secondary metabolites, and they can also be used to purify and take up nutrients from wastewater [1].
In the development of algal products, one of the major targets is to be select and optimise a suitable culture nutrient medium [2]. The choice of culture media mainly depends on several factors including the chemical composition of media [3 & 4]. The maintenance of algal strains is dependent on the growth media and culture parameters. Thus, growth of algae is a function of many factors, including nutrients, pH and salinity of the culture media and the cultural parameters like temperature and light [5]. Artificial media with known chemical composition is used to stimulate diverse nutrient requirement for the growth of several algal species. There are a number of media recipes which is commonly used for the microalgae cultivation [3 & 6]. A common approach to determine the factors for optimal biomass production is to start the algal culture with a known medium and associate it with other elements that optimize microalgae's growth and nutritive value [7].
In recent years it has been suggested that microalgae of the Chlorophyceae class could be excellent reservoirs of nutraceuticals, because they contain polyphenols, chlorophyll, β-carotene, ascorbic acid, lycopene, α-tocopherol, xanthophylls, and poly unsaturated fatty acids [8]. Nutraceutical industry is mainly dominated by two Cyanophycean genera, *Spirulina* and *Aphanizomenon* and the Chlorophycean *Chlorella* [9]. *Chlorella* is the most popular and cultivated eukaryotic green microalga, as it is used as feed supplement. It is also used in the pharmaceutical and cosmetics industry [10]. It contains proteins, carotenoids, lipids, polysaccharides, vitamins, antioxidants and minerals [11]. *Chlorella* sp can be easily cultivated in simple conditions, producing enormous quantities of biomass in little time. It needs only water, CO2, light, and a small quantity of minerals [12]. One of the commercially successful green microalga is the astaxanthin rich *Haematococcus pluvialis*. *Haematococcus pluvialis* synthesizes and accumulates high levels of astaxanthin under stress conditions such as high light irradiance and nitrogen starvation [13, 14 &15].
So, considering the high importance of microalgal culture, three species of Chlorophycean members such as *Chlorella ellipsoidea*, *Chlorella vulgaris* and *Coelastrum* sp were selected for culture in different media to evaluate the best culture media. The aim of this work is to evaluate the effect of four different defined media such as Bold's Basal Medium, CHU10, Walne's and 3N BBM+Vitamin medium on growth parameters of selected algal strains.

2. Materials and Methods
2.1 Sample collection
Samples were collected from selected locations of Vembanad estuary during the Pre- Monsoon period (February to May) of 2019.

2.2 Isolation and Establishment of monoculture
The collected microalgal samples are enriched by adding Chu 10 medium. After initial cultivation of the mixed cultures, microalgae are subjected to isolation by serial dilution and agar streak plate method. The individual cells were picked up and transferred into the same media in 250 ml conical flasks. Culture broths were shaken manually for two times per day. Purified algal species were identified on the basis of morphological features using a Biolinkz M2000

light microscope. Systematic identification up to the species level was carried out with the help of standard and available literature [16 & 17].

2.3 Effect of culture media

Selected algal species were grown on various artificial media in order to check which of the medium was able to support the best growth of algae. In order to find out the best culture medium, screened algal species were cultured in four different culture media such as Bold's Basal medium, Chu 10, Walne's and 3N BBM+Vitamin medium. Before experimental setup all the prepared media were sterilized in an autoclave at 15 psi, and 120 ◦C temperature. The pH of the media were adjusted by using 1N NaOH and 1N HCl prior to autoclaving. Autoclaved media were carefully transferred into sterilized culture vessels. The microalgal cultures were maintained in the algal culture room under controlled conditions of temperature (25°C) and light (1 k lux). An initial inoculum was added to each flask and the growth was monitored by taking cell counts every day at 11 a.m. For counting, a one ml sample was removed from each culture and the cells were counted with the aid of a Neubauer haemocytometer. The growth rate was noted for 30 days [18].

3. Results

Three morphologically different strains were isolated from Vembanad estuary. The morphology of isolated strains were studied under light microscope, and identified as *Chlorella ellipsoidea, Chlorella vulgaris* and *Coelastrum* sp.

3.1 Growth of *Chlorella ellipsoidea*

Culture of *Chlorella ellipsoidea* started with 0.05 x 10^6 cells /ml, attained maximum cell density of 5.8×10^6 cells/ ml within 27 days in 3NBBM+V Medium. Exponential phase was attained from the 18th day of culture. During the culture period, the range of cell density of *C. ellipsoidea* in 3N BBM +Vitamin medium ranged from 0.05 to 5.8×10^6 cells ml with an average cell density of 1.20±0.588 ($\times10^6$) cells/ml. The maximum growth was observed in 3N BBM+Vitamin medium followed by Walne's, BBM, and Chu 10, medium. The growth rate of *Chlorella ellipsoidea* expressed in cell counts in different media is illustrated in Fig.1 and 4.

3.2 Growth of *Chlorella vulgaris*

The culture of *Chlorella vulgaris* started with an initial cell density of 0.12 x 10^6 cells /ml, attained a maximum cell density of 26.7 x 10^6 *cells* /ml within 18 days in Bolds Basal Medium. The maximum growth was observed in BBM medium followed by Chu 10 (19.59 $\times10^6$ within 18 days), 3NBBM+Vitamin medium (13.3×10^6 within 18 days) and Walne's medium (6.80×10^6 within 15 days). The average cell density in BBM was 14.0±3.12 (10^6) cells /ml. The growth rate of *Chlorella vulgaris* expressed in cell counts in different media is illustrated in Fig. 2 and 5.

3.4 Growth of *Coelastrum* sp

Cell density of *Coelastrum* sp ranged from to 0.77×10^6 cells/ ml to 8.72×10^6 cells/ml, during the culture period in Chu 10 medium. Exponential phase was attained 18th day from the starting of culture in Chu 10 medium and after that from stationary phase cell density began to decline towards death phase. The maximum growth was observed in Chu 10 medium followed by 3N BBM+V medium (2×10^6 cells/ ml within 30 days), BBM (0.86×10^6 cells/ ml within 15 days) and Walne's medium (0.71×10^6 within 18 days). The average cell density in Chu 10 medium was 3.61±1.14 (10^6) cells /ml. The growth rate of *Coelastrum* sp expressed in cell counts in different media is illustrated in Fig. 3 and 6.

Table 1: Cell densities (x10^6cells/ml) of *Chlorella ellipsoidea* cultured in 4 different media for a period of 30 days

Culture medium	Duration of culture in days										
	3	6	9	12	15	18	21	24	27	30	Mean ±SD
Walne's	0.03	0.07	0.29	0.50	0.85	0.90	1.50	1.61	1.91	1.0	0.86±0.216
Chu 10	0.32	0.16	0.24	2.8	0.65	0.58	0.50	0.48	0.45	0.30	0.64±0.255
BBM	0.02	0.15	0.38	0.62	0.88	1.90	1.70	1.50	0.62	0.50	0.829±0.216
3NBBM+V	0.05	0.08	0.13	0.19	0.33	1.20	1.50	2.20	5.80	0.60	1.20±0.588

Table 2: Cell densities (x 10^6 cells/ml) of *Chlorella vulgaris* cultured in 4 different media for a period of 30 days

Culture medium	Duration of culture in days										
	3	6	9	12	15	18	21	24	27	30	Mean±SD
Walne's	0.08	0.24	1.34	16.7	6.80	6.20	5.80	5.20	4.80	4.00	5.16±1.58
Chu 10	0.59	3.8	9.4	14.3	19.6	19.59	15.4	12.7	11.5	10.0	11.6±2.03
BBM	0.12	1.66	2.05	15.9	18.9	26.7	21.6	20.5	18.2	15.0	14.0±3.12
3NBBM+V	0.06	0.98	1.41	6.09	10.3	13.3	7.9	7.50	6.00	5.80	5.93±1.39

Table 3: Cell densities (x 10^6 cells/ml) of *Coelastrum* sp cultured in 4 different media for a period of 30 days

Culture medium	Duration of culture in days										
	3	6	9	12	15	18	21	24	27	30	Mean±SD
Walne's	0.25	0.28	0.54	0.61	0.70	0.71	0.65	0.40	0.38	0.20	0.47±0.064
Chu 10	0.77	2.28	2.38	7.14	7.49	8.72	6.46	0.34	0.32	0.28	3.61±1.14
BBM	0.15	0.24	0.38	0.80	0.86	0.77	0.76	0.50	0.40	0.30	0.51±0.08
3NBBM+V	0.03	0.10	0.29	0.67	0.80	0.90	1.19	1.20	1.50	2	0.86±0.20

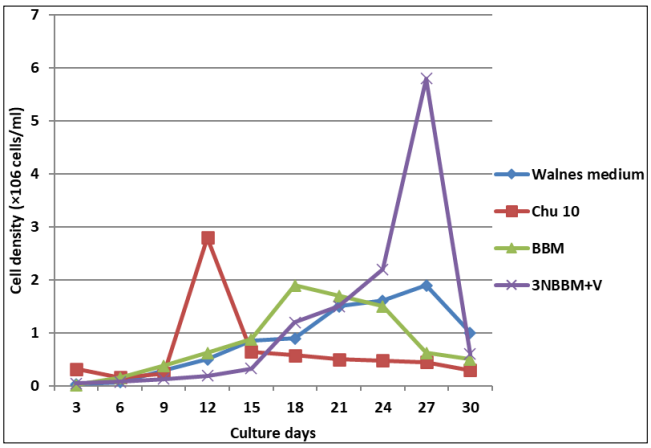

Fig 2: Growth of *Chlorella ellipsoidea* in 4 different culture media

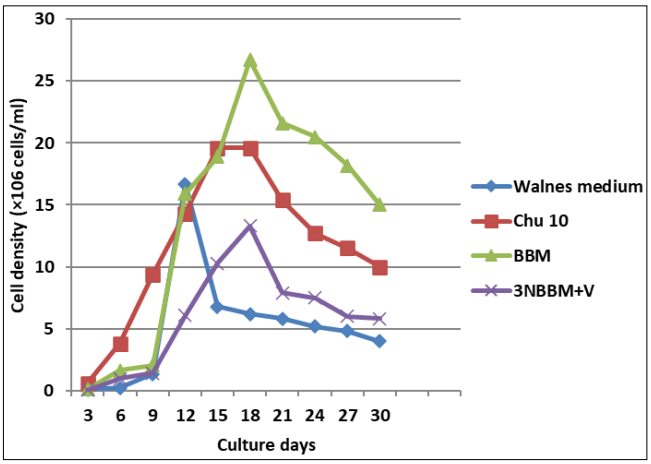

Fig 5: Growth of *Chlorella vulgaris* in 4 different culture media

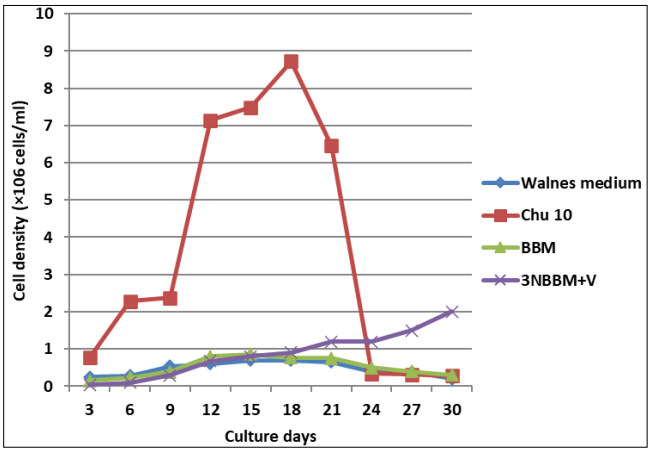

Fig 6: Growth of *Coelastrum* sp in 4 different culture media

4. Discussion

Microalgae are excellent reservoirs of wide range of biologically active compounds. Many researchers utilized unialgal cultures for various applications starting from therapeutics to biodiesel production [19]. So, it is necessary to find the optimum medium for their biomass accumulation. The present study focused on the standardization of culture media for three isolated green algae such as *Chlorella ellipsoidea, Chlorella Vulgaris and Coelastrum* sp. The growth of three different microalgae in different culture media was conducted by counting algal cells under the microscope using a haemocytometer. The growth was tracked for 30 days.

In the present study culture of *Chlorella ellipsoidea* started with a cell density of 0.05 x 10^6 cells /ml, attained maximum cell density of 5.8×10^6 cells/ ml within 27 days in 3NBBM+V medium. The average cell density was 1.20±0.588 (×10^6) cells/ml. Several studies on *Chlorella ellipsoidea* have been focussed on the standardization of culture media and biomass accumulation [20, 21, 22 &23]. An investigation on the growth of *Chlorella ellipsoidea* in various organic and inorganic media was carried out [20] and maximum cell density was obtained in medium supplemented with pulsed bran (0.18 to 4.38 ×10 ^{6}cell ml). The earlier workers have reported [22 & 23] that the growth of *Chlorella ellipsoidea* were significantly higher in Bold Basal Medium supplemented with riped bean seed powder at the concentration of 1.0g/l and also in media supplemented with organic whole pulse powder extract (30.69×10^6 cell ml).A significant growth rate was attained when *Chlorella ellipsoidea* is cultured in a fertilizer factory effluent media [24]. These results of cell densities are much higher in comparison to those of the present experiment. Addition of organic and inorganic supplements to the culture media may enhance the growth of *Chlorella ellipsoidea*.

In the present experiment, Bold's Basal Medium gives the maximum cell density for *Chlorella Vulgaris* (26.7 x 10^6 cells /ml) comparing to the other media. The average cell density in BBM was 14.0±3.12 (10^6) cells /ml. Prior studies on *Chlorella vulgaris* state the significance of Bold's Basal Medium on growth and biomass production [25,26,27,28& 29]. An investigation on the effect of various growth medium compositions on *Chlorella vulgaris* to enhance its growth and lipid production using batch culture conditions were carried out [25]. Thirteen different growth media were being tested in culture tubes and maximum cell density was recorded for the Bold's Basal Medium (5.68 × 10 ^{6}cells/ml). In a similar observation [26], the species of *Chlorella* and *Monoraphidium* exhibited maximum growth in Chu 10 medium and *Scenedesmus* sp shows the maximum growth in Bolds Basal Medium. Other researchers have also observed that *Chlorella vulgaris* cultivated in BBM shows higher protein and lipid production [27&28]. Higher growth rate and biomass accumulation for *Chlorella vulgaris* was obtained in BG 11medium [6] and also in BBM media supplemented with urea and glucose [29].

In the current study *Coelastrum* sp gives maximum growth in Chu 10 medium with cell density of 8.72× 10^6 cells/ml. Earlier works reported [30] the isolation and culture of *Coelastrum* sp. TISTR 9501RE in BG 11 medium for 14 days. As the previous works on the cultural parameters of *Coelastrum* sp were limited, further studies have to be performed for their further standardization. A clear understanding of the nutritional requirement of these microalgae is therefore a prerequisite for determining the technique of culturing and maintaining the algae for a long period and biomass accumulation.

5. Conclusion

The present work investigated the effect of four different medium namely Bold Basal medium, Chu 10, Walne's and 3N BBM+Vitamin medium on three different microalgae. All the three organisms shows varied growth pattern in different culture media. Present experiment clearly shows that 3NBBM+V Medium gives maximum cell density for *Chlorella ellipsoidea.* The cell densities in Bold's basal

media were significantly higher in the case of *Chlorella vulgaris* than those of other media. Chu 10 medium favour's the growth of *Coelastrum* sp. The cultivation of these microalgal strains in the optimised media may enhance the total biomass of the algae concerned. A further study with combination of other organic materials is required to find out the cost-effective media for mass production of the algae selected in this study.

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