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Determination of Suitable Growth Medium for *Chlorella Minutissima*

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Abstract

The present investigation was undertaken to check the best suitable medium for high biomass production rate of genus *Chlorella minutissima*. Preliminary investigations undertaken with three widely used media i.e. CHU-10 medium, BG-11 medium, and Allen and Arnon medium. Growth measurements revealed that BG-11 medium enhance biomass production significantly as evident by cell count number and chlorophyll a content estimation.

Keywords: Microalgae, *Chlorella minutissima*, Growth measurement, Chlorophyll a, Growth medium.

Introduction

The last few decades have seen a growing interest in using microalgae as potential tool for carbon sequestration [1]. *Chlorella minutissima* has been one of the most targeted microalgae and been found to tolerate up to 50% CO₂, able to grow well in presence of low concentrations of SO₂ and NO_x, indicating that it can be used for direct CO₂ fixation from actual flue gas [2] that is unsuitable for higher plants. The genus *Chlorella minutissima* is a unicellular eukaryotic alga with a fast growth rate and is a widely employed microalga for biomass production. *Chlorella minutissima* belong to division chlorophyta, has chlorophyll *a* and *b*, spherical in shape, lack flagella, and synthesizes starch like plants. *Chlorella minutissima* is rich in amino acids and polyunsaturated fatty acids, which makes it potentially useful in health foods and pharmaceuticals too [3]. Therefore, cultivation of algal biomass provide dual benefit, production of various useful products and at the same time save our environment from air and water pollution [1]. Factors such as temperature, irradiance and, most markedly, nutrient availability have been shown to affect the growth as well as physiology in several algae [4]. *Chara corallina* was reported to grow best in Allen and Arnon's medium [5].

The objective of the present study was to perform a comparative study for determination of a suitable growth medium for *Chlorella minutissima*. It is a preliminary investigation. Based upon growth measurements viz. cell count and Chlorophyll *a* content, best medium was selected.

Materials and methods

Culture Media

Chlorella minutissima was tested for its growth in different culture media like CHU-10 medium [6], BG-11 medium [7], and Allen and Arnon medium [8]. Equal amount of culture is distributed in all the test tubes containing media.

Maintenance of the cultures

Stocks as well as routine cultures were kept in the culture room maintained at 27±2°C and 1400 lux light intensity. The cultures were illuminated daily by 40W fluorescent tubes for 14 hours light cycle. All the culture vessels were manually shaken for 2-3 times daily to keep the cells suspended in the liquid medium.

Growth measurements

1. Counting of Algal Cells

Growth measurements were carried out by counting the number of algal cells in each of the experimental flask on the 3rd, 6th and 9th day after inoculation. For counting, the sample/flask was agitated vigorously so as to distribute the organisms evenly. The cell number was counted by using the Sedgwick –Rafter plankton counting slide on a light microscope. Counting of plankton was done with the help of “Sedgwick–Rafter counting cell” as per the procedure given by Wetzel and Likens [9]. Sedgwick –Rafter is a 50mm long, 20mm wide and 1 mm deep cell unit with a volume of 1cm³ or 1ml. The counting cell unit and the cover-slips were cleaned by using rectified spirit. Exactly 1ml of the sample was transferred aseptically onto the cell unit using a pipette and a clean cover-slip was placed over it. Samples

were allowed to settle in the counting chamber for 3–5 min prior to enumeration. More than ten fields of view were randomly selected across each slide and repeated three times.

As it was inconvenient to count the algal cells in the experimental flask undiluted; so the sample was diluted in distilled water (depending upon the concentration of algal cells) so as to make the counting easy.

The number of microalgal cells per ml was calculated as:

$$\frac{\text{Number of organisms counted in all replicates} \times 1000}{\text{Dilution factor}}$$

$l \times b \times d$ of cell unit X number of replicates counted

2. Estimation of Chlorophyll a

Growth in terms of Chlorophyll a was estimated by the method as described by Mackinney [10]. Chlorophyll a content was determined spectrophotometrically on the 0 day, 3rd, 6th and 9th day of inoculation in media. A known volume of algal culture (15ml) was centrifuged at 2500rpm and the pellet was washed with distilled water and re-suspended into culture tubes containing 10 ml of 90% (v/v) methanol. The tubes were covered properly to prevent the evaporation of methanolic extract. After that the tubes were placed in a water bath at 60°C for extraction of chlorophyll-a. When pellets become decolorized, samples were taken out from water bath and cooled at room temperature. The volume of the sample was adjusted up to original volume by adding 90% methanol. The samples were centrifuged and

supernatant were taken for reading at their absorbance at 650 nm and 665 nm with the help of Systronics-108 spectrophotometer. The chlorophyll a content was calculated by using following formula:

$$\text{Total chlorophyll a } (\mu\text{g/ml}) = 2.55 \times 10^{-2} \times \text{OD}_{650} + 0.4 \times 10^{-2} \times \text{OD}_{665}$$

By knowing the concentration of the pigment value, permit value of the sample was calculated by the following formula:

$$\text{Chl}_a = \frac{\text{Chl}_a \times \text{Extract volume in litres}}{\text{Vol. (in litres) of watersample}}$$

Results and discussion

Figure 1 shows the growth pattern of algal cells in three different growth media. It was found that *Chlorella minutissima* can be grown in all three culture media (Table 1), viz., Allen and Arnon’s medium, Chu-10 medium and BG-11 medium but growth is seen to be the best in BG-11 medium culture medium in comparison with the other two culture media. Readings were taken on zero day, 3rd day, 6th day and 9th day respectively. Number of cells steadily declined in case of Allen & Arnon medium while in case of Chu-10 medium it came to stagnant stage after moderate growth. Growth comes poor in case of Allen & Arnon medium might be due to the inhibitory effect of high salt concentration. *Chlorella minutissima* shows its best growth in BG-11 medium as evident by cell count number (Table 1). Growth reaches at its peak in terms of cell count number on 6th day then it slowly decline with passage of time.

Table 1 Growth (Cell number/ml) of *Chlorella minutissima* in different media

Number of days	Allen & Arnon	Chu-10	BG-11
0 Day	68 X 10 ⁴	45 X 10 ⁴	66 X10 ⁴
3 rd day	40X 10 ⁵	46 X 10 ⁵	62 X 10 ⁵
6 th day	18 X 10 ⁵	51 X 10 ⁵	25 X 10 ⁶
9 th day	31 X 10 ⁴	16 X10 ⁵	12 X 10 ⁶

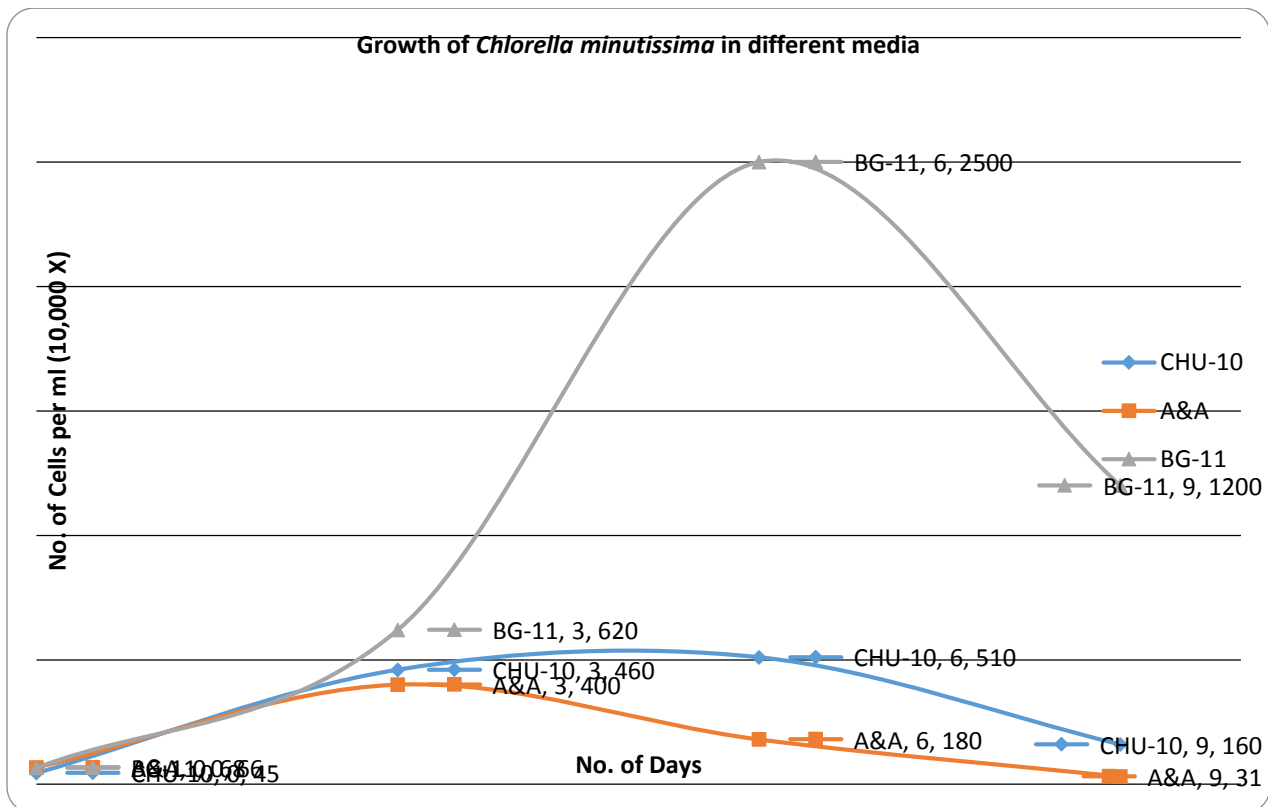


Figure 1 Growth curves of *Chlorella minutissima* in three different media, based on cell counts per ml. Each point represents the mean of three replicates \pm SD ($n = 3$).

Similar tendency was observed for variation in Chlorophyll *a* content from all the three culture media (Figure 2). Readings were taken on zero day, 3rd day, 6th day and 9th day respectively. *Chlorella minutissima*

shows its best growth in BG-11 medium in terms of chlorophyll *a* content. Chlorophyll *a* content in BG 11 increases continuously with time.

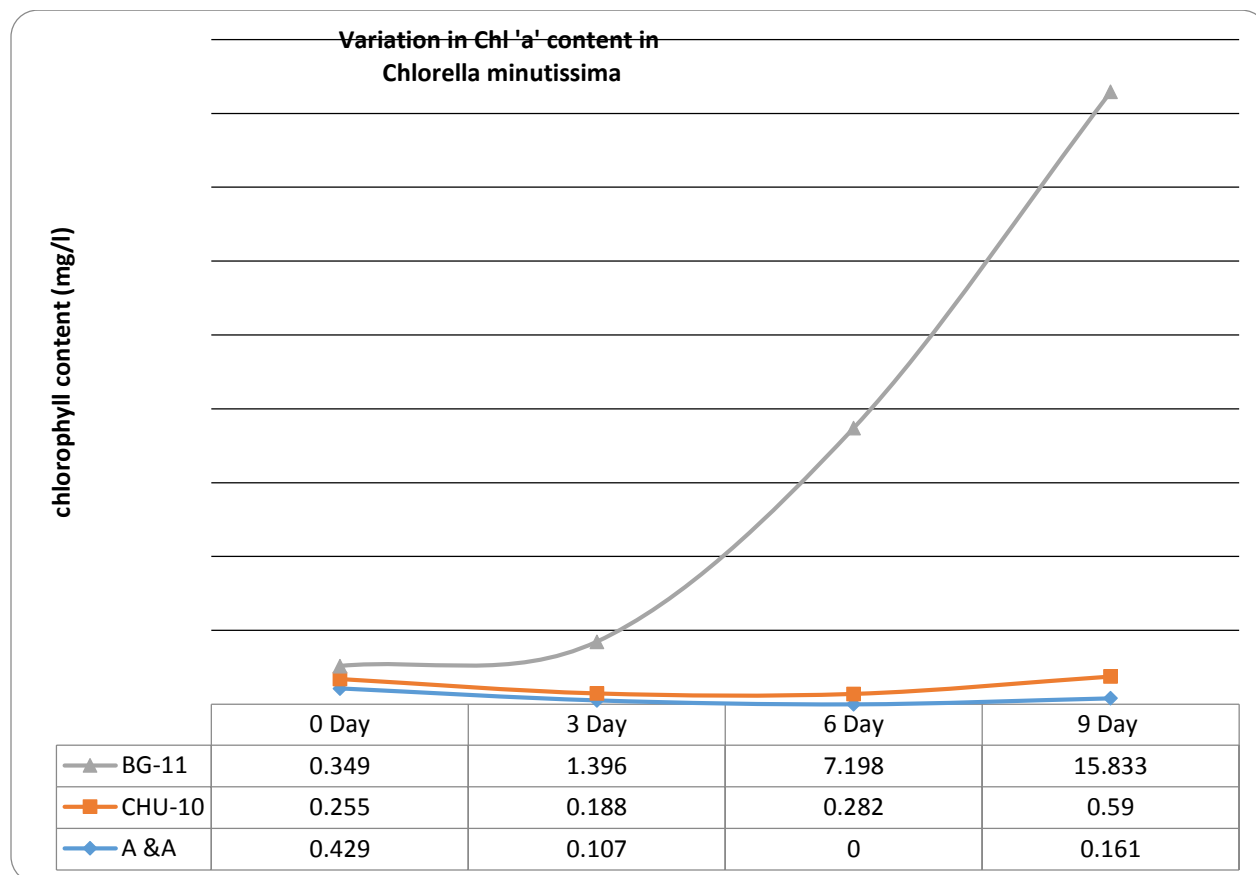


Figure 2 Growth curves of *Chlorella minutissima* in three different media, based on chlorophyll a estimation. Each point represents the mean of three replicates ± SD (n = 3).

These results clearly showed that BG-11 medium is best to grow *Chlorella minutissima* among three media used. It can greatly help to maintain mass amounts of *Chlorella minutissima* in outdoor cultivations. Now the further experiments regarding standardization of other parameters of growth for *Chlorella minutissima* will be conducted in BG-11 medium. In the light of above experiments it is evident that *Chlorella minutissima* is appropriate raw material carbon sequestration.

Conclusion

Chlorella minutissima showed different response against the type of media. Among the three algal media studied, *Chlorella minutissima* shows its best growth in BG-11 medium. Growth comes poor in case of Allen & Arnon medium because of high salt concentration. While in Chu-10 medium *C. minutissima* shows moderate growth. This study reveals the promise of using *Chlorella minutissima* for biomass production and carbon sequestration.

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