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Case study

AN EXPERIMENTAL STUDY: USING PLANT FERTILIZER AS A POTENT CULTURE MEDIA FOR *CHLORELLA VULGARIS*

G.P.SINGH AND NAMITA SIKARWAR

Algal Biotectnology Lab, Department of Botany, University of Rajasthan, Jaipur, Rajasthan. Email: gajendra221@hotmail.com

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ABSTRACT

During growth, living organisms absorb chemical elements from their environment in ratios as they occur in their tissues. These elements are absorbed as small molecules or as free ions, potentially affecting the relative ionic composition of their medium. To avoid these changes in the medium composition, the elements in culture media should be available in the same ratios as in which they occur in biological material. This paper showed how, by using liquid plant fertilizer as culture media was designed which approximate the average elemental composition of biological material. A preparation of green algae media such as Juller's media (as standard) and different concentration of liquid plant fertilizer (v/2V,v/4V,v/6V and v/8V) A comparative study was carried to estimate optical density, cell count and dry mass (Growth kinetics) by using spectrophotometer, hematocytometer (Neubauer, improved) and dry weight method respectively. Results obtain showed maximum growth kinetics in *chlorella vulgaris* using liquid plant fertilizer media.

Keywords: Sectrophotometer, Liquid Plant Fertilizer Media, *Chlorella vulgaris*

INTRODUCTION

Algal nutrient solutions are made up of a mixture of chemical salts and water. Sometimes referred to as "Growth Media", nutrient solutions (along with carbon dioxide and light), provide the materials needed for algae to grow. Nutrient solutions are designed specifically for use in aquatic environments and their composition is much more precise. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the marine water.

Algae has a multitude of uses and contains a number of different bioactive compounds such as; chlorophyll, which acts as a detoxifier and possibly an anticancer agent (Hans,1992), Carotenoids, which also have the widest commercial application in improving the health and fertility of cattle (Barawitzka,1998), C-Phycocyanin, which is widely used in the pharmaceutical field as it is known to have antitumor, antioxidant and antiproliferating activity,(Ramay et al.,1998), as well as Phycoerythrin, which has an application as a natural dye as well as in fluorescence microscopy ,(Glazer and Stryer, 1984). These may be used as pigment or colourants in the food industries as well for cosmetics,(Borowitzka,1994)

An experiment was conducted to know the growth conditions of algae on different concentration of plant fertilizer keeping jullers as starndrad media. Some of the investigators who worked are; Castrday et.al,(1984); lustingman et.al (1995), Price and Morel (1990) on manganese, cobalt & zinc, Prask and Plocke (1971), De-fillippis et. al. (1981) and Lu et.al.(2000) on mercury.

A comparative study was carried to estimate optical density, cell count and dry mass (Growth kinetics) by using spectrophotometer, hematocytometer (Neubauer, improved) and dry weight method respectively of *chlorella vulgaris* to optimize the culture condition in the laboratory.

MATERIAL AND METHODS

Microorganism and culture condition

The unialgal culture of chlorella vulgaris was collected from different areas of Jaipur. The cultures will be developed in the laboratory. In order to find out the best culture media different concentration of plant fertilizer are used such as v/2V,v/4V,v/6V,v/8V.the cultures were grown with photoperiod of 12 hours light/dark provided by white flourescent lamp at a light intensity of 2500 lux and temperature of 25 ±20C. influence of the different media concentration was given in stepwise individual experiment.

Cultivation

For the cultivation of *Cholrella vulgaris* four days old prepared inoculums of unialgal culture was added to three sets of 500 ml conical flask containing 250 ml sterizied Jullers media. To observe the influence of different media on growth and pigment concentration accumulation, the culture of *Chlorella vulgaris* were grown of 25 ± 2 ^{0}C at 2,500 lux light intensity.during the process of growth, the cultures were shaken thrice a day to avoid clumping and accelerate the growth process.

Analytical Methods

Growth will be followed through optical density of the culture. the optical density of the green algal suspension at 560nm, as recommended by Wetherel(1961).Culture grown under optimum culture condition will be analysed biochemically for their biopigments by using standard methods suggested by Parson and Strickland (1965) for chlorophylls will be carried out. Cultures were analyzed for their growth and pigments contents every 5th day.

Statistical analysis

Results of the analyses were compared by one way analysis of variance (ANOVA). The significance between pairs of variable means were analysed using least significant difference (LSD) test at 5% level of significance.

RESULTS

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 v/4V medium shows maximum growth followed by v/6Vmedium, Juller's medium, v/8V medium and minimum growth was observed in v/2V medium Optical density and cell count clearly indicated that the best growth of C. vulgaris was obtained in modified v/4V medium as compared to that in other media. OD and CC had increased by 3.4 times and 3.2 times of the initial record respectively, after a period of five weeks (Table -1 and Table -2). v/6V medium was next to modified v/4V solution in promoting the growth of C. vulgaris. Growth was increased about 3.12 times in terms of OD and 2.8 times

in terms of CC. In Juller's medium OD and CC were increased 2.68 and 2.1 times, respectively, of the initial record. In v/8V medium OD was increased 2.49 times and CC was increased 2.0 times the initial record. v/8V medium proved to be insufficient in supporting the growth of C. vulgaris as has been observed through OD as well as CC (Graph 1 and Graph 2), which increased up to 1.2 and 1.8 times the initial record, respectively. 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 v/4V medium i.e. 2.13% and 0.59%, respectively, after a period of five weeks, followed by 2.01% and 0.62% in v/6V medium, 1.84% and 0.70% in Juller's medium, 1.52% and 0.41% in v/8V medium and minimum Chl-a and Chl-b content were found in v/8V medium i.e. 1.66% and 0.41%, respectively (Graph 3 and Graph 4). The growth rate of the algae seemed to be directly associated with morphological configurations. The normal morphological configuration was observed in modified v/4V 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. v/6V 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. v/8V 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 v/8V 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 v/4V 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 v/4V will result in a faster rate and greater amount of growth of many of the algae.

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. K2HPO4 was the source of phosphate in modified v/4V 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, 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(NO3)2 4H2O 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. As nitrogen deficiency develops the amount of chlorophyll in the cells decreases faster than the nitrogen content in *C. vulgaris* cultures. MgSO4 was the source of magnesium in modified v/4V 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. 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. The process of multiplication requires a larger concentration of magnesium in the medium than does the production of cell material.

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. Ferric citrate and citric acid combination was the source of iron in modified v/4V medium by substituting an organic source of iron, ferric citrate, for the ferric chloride. This improvement is due to increased iron availability and an equal amount of citric acid with the ferric citrate stabilized the concentration of reactive iron in the nutrient solution. It has mentioned above that the water body has pH 7.3 from where the algae have been isolated. The pH of modified v/4V 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 7.528 and some scientists maintained unialgal and axenic cultures of *C.vulgaris* in modified v/4V medium in their research work but they had not mentioned the impact of this medium on growth and morphology of *C. vulgaris*. The green alga *Botryococcus protuberans* has shown enhanced growth rate in the modified v/4V medium.

The performance of different species in different media may also be conditioned by the previous history of the cells. In previous studies it has been shown that modified v/4V medium support the growth of blue green algae. However, in our experiments, the modified v/4V medium supports the growth of green alga, *Chlorella vulgaris*.

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