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Effect of Different Growth Media on the Cell Densities of Freshwater Microalgae Isolates

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Abstract: *The effect of BMM and BG-11 medium on the cell density of two economically important microalgal isolates (Chlorella sp. and Scenedesmus sp.) from Abakaliki fresh water body was investigated. The isolates were incubated in the growth media for the period of 384hours (16 days) during which biomass concentration was obtained through microscopic examination of cells at 48th hour interval, using haemocytometer. The culture broths turned green during cultivation an indication that the isolates were green algae. All the isolates showed varied growth pattern in different culture media with both cells optimal in BMM. This suggested that Bristol Modified Medium is a better medium when compared to Blue- Green Medium in cultivation of freshwater microalgae for cell density yield.*

Keywords: *Chlorella sp, Density, Freshwater, Growth Media, Scenedesmus sp.*

I. Introduction

Microalgae are generally defined as all photosynthetic eukaryotes (with the exception of land plants) and prokaryotic cyanobacteria [1]. Unicellular microalgae are the fastest growing, photosynthesizing organisms and can complete an entire growing cycle every few days if adequate amounts of sunlight, water, carbon dioxide, and nutrients are available [2]. Microalgae are microscopic algae, typically found in freshwater and marine systems living in both the water column and sediment [3]. They are unicellular species which exist individually, or in chains or groups. Depending on the species, their sizes can range from a few micrometres (μm) to a few hundreds of micrometres. Unlike higher plants, microalgae do not have roots, stems and leaves. They are specially adapted to an environment dominated by viscous forces [4].

Algae are nonfood resources that are amenable for cultivation on non-arable land using saline water and wastewater. Temperature, light intensity, amount and type of nutrients, amount of CO_2, and pH are the key factors influencing algal growth. Algae represent a very diverse and heterogeneous complex of organisms belonging to many different phyla, and characterized by very different physiological attributes. A direct consequence of this great diversity is that different species of algae have very different growth requirements. Therefore, location is a key determining factor for the selection of microalgae strains that can be used to produce biomass [1]. Of the many algal strains available for the investigation of growth rates and bio-fuel production potential, the ideal strain will likely be different for each location, particularly if outdoor cultivation is utilized. The environmental conditions of a specific area can greatly influence microalgae populations and their growth dynamics. Therefore, the most logical approach is to screen for highly productive strains with maximum lipid contents at selected sites, and optimize the growth conditions for large-scale cultivation [5].

Many microalgae possess few morphological traits that are useful for species characterization, leading to the possibility of numerous cryptic species. For example, some of the most commonly reported microalgae are coccoid organisms, often referred to as "little green balls" [6]. These organisms are extremely difficult to identify because of their small size and simple morphologies. As a result of these, molecular techniques are employed for more proper classification and identification of algae to their species level [6].

The effects of recent events such as climate change, environmental pollution and chronic diseases can be mitigated with microalgae and their products. These products include polyunsaturated fatty acids, beta-carotene, astaxathin and other biomolecules that are produced during metabolic changes in algal cell. The nature and amounts of these biomaterials formed are determined basically by the nutrients and the culture condition (such as temperature, light and pH). Microalgae require both macronutrients and micronutrients for their growth and metabolic function. Types of algal medium are determine by the chemical composition of these nutrients. Several growth media abound for the growth of algae, even it is important to know which medium is best for optimum growth of the organisms [1 and 7].

Our goal in this study was to evaluate the most suitable medium between Blue green medium (BG-11) and Bristol modified medium (BMM) for cultivation of freshwater microalgal isolates. The objective of this present investigation was to analyze the growth and sources of algae in order find out which growth media is most suitable for maximum cultivation.

II. Materials And Methods

Sample collection

Water samples with visible microalgae population were collected from fish pond at CAS campus, Ebonyi State University (EBSU) and stream along water-works road Abakaliki. Collections were made from the top and bottom of the water at each location, with the goal of determining the dominant microalgae species in each area. The field samples were collected in 50 ml tubes and maintained at room temperature condition while transferring to laboratory. The pH of the water samples were taken using a pH meter (SP60, Germany), that of the fish pond was 7.8 whereas that of stream was 8.2.

Antibiotic Treatment of Cultures

The cultures were treated with little quantity of 250mg of chloramphenical that was necessary for population genetics, molecular biology or bio-product screening for their mode of action, either as inhibitor of cell wall synthesis or of cell growth via inhibition of protein synthesis in bacteria.

Growth Media

In order to test for preferential growth of isolates, the samples were subjected to two different media of varying nutrient composition as stated.

I Blue- Green Medium (BG-11): The medium contains the following chemicals;

$NaNO_3$ 1.5 g, $K_2HPO_4.3H_2O$ 0.04 g, $KH_2PO_4.3H_2O$ 0.2 g, disodium EDTA 0.001 g, Fe ammonium citrate 0.001 g, citric acid 0.006 g, Na_2CO_3 0.02 g and 1 ml of trace metal solution per litre, pH 7.3.

The trace metal solution contains H_3BO_3 2.85 g, $MnCl_2$. $4H_2O$ 1.8 g, $ZnSO_4.7H_2O$ 0.02 g, $CuSO_4.5H_2O$ 0.08 g, $CoCl_2$.$6H_2O$ 0.08 g and $Na_2MoO_4.2H_2O$ 0.05 g per litre

II Bristol Modified Medium (BMM): The concentrations of nutrients in this medium (g/400ml of sterile water) were: 10g $NaNO_3$, 1g $CaCl_2{\cdot}2H_2O$, 3g $MgSO_4{\cdot}7H_2O$, 3g K_2HPO_4, 7g KH_2PO_4 and 1g NaCl. One drop of 1% ferric chloride solution and 40ml of Pringsheim's soil-water extract were also added to medium.

The growth media were prepared based on their compositions, 200ml BG-11 and BMM transferred into 500ml Erlenmeyer flasks and sterilized at 121^oC for 15min and cooled to room temperature (25^0C) prior to use. Microalgae samples were inoculated in these two media. The cultures were gently shaken in order to accelerate the algal growth. All tests were carried out in triplets [8, 9 and 10].

Cultivation and Harvesting

Each algal culture sample was monitored every two days for cellular growth rates by counting the cell concentration using a Haemocytometer (L 8800, Hitachi High Technologies Japan), with a cover slide for two weeks. The microalgae species were harvested by dispensing a normal quantity of the algal culture into micro eppendorf tube and spun for 15min at 1200rpm for three consecutive times and preserved in the refrigerator for further use [11].

Morphological Identification

Microalgae in cultures were examined microscopically and photographed with "Leica micro photographic unit". Cells identification was based on colour, shape, size, and cell dimensions [12 and 13], using the Nikon Eclipse E800 microscope (Nikon Inc., Tokyo, Japan) with the DXM1200 digital camera.

Analytical Method

Total cell counts were microscopically determined with a Neubauer counting chamber (depth= 0.1mm; area = $1/400mm^2$) (Weber English). Number of cell/ml = $n \times 4 \times 10^6$ cell/ml.

Where n = average number of cells per well.

III. Results

After cultivation for 2-4 weeks, observations were made under a light microscope. Isolates were selected based on rapid growth, morphlogical and color diversity. They were tentatively identified as *Chlorella* sp. and *Scenedesmus* sp. respectively.

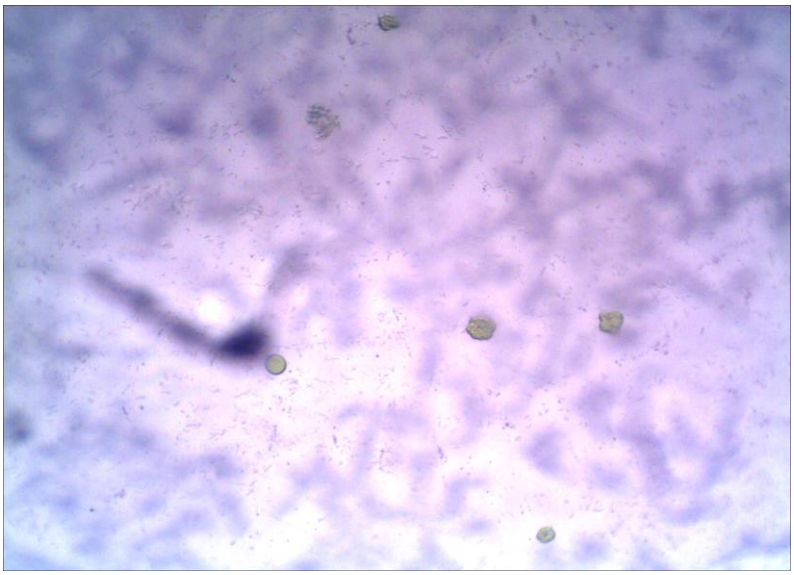

Fig. 1: Microphotograph of *Chlorella* sp

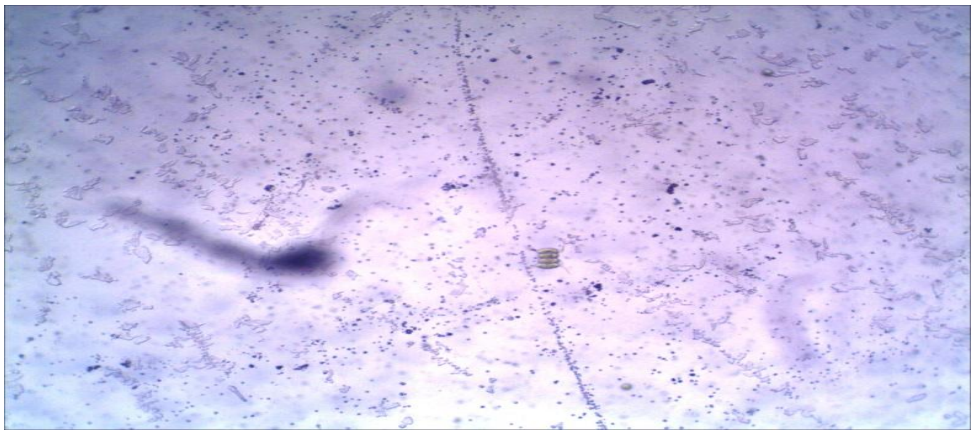

Fig.2: Microphotograph of *Scenedesmus* sp

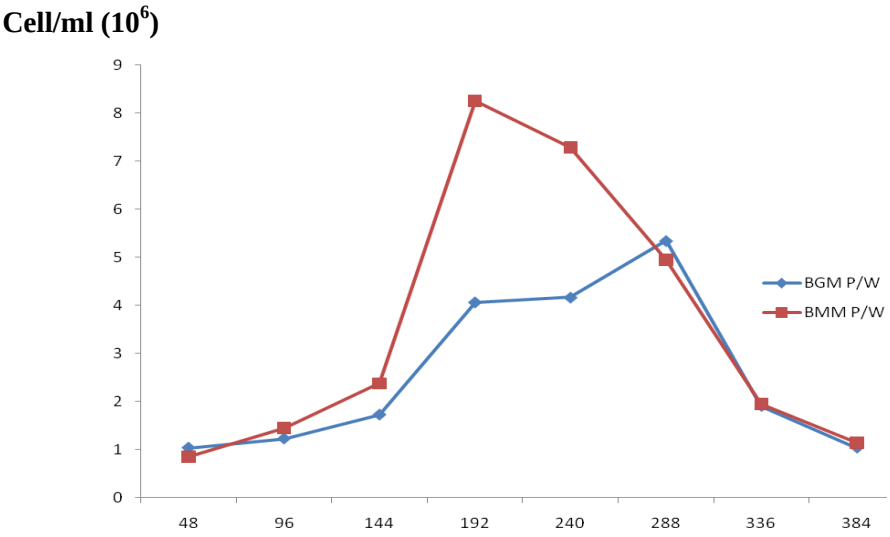

Fig. 3: Growth Curve of *Chlorella* sp. 48-384hours

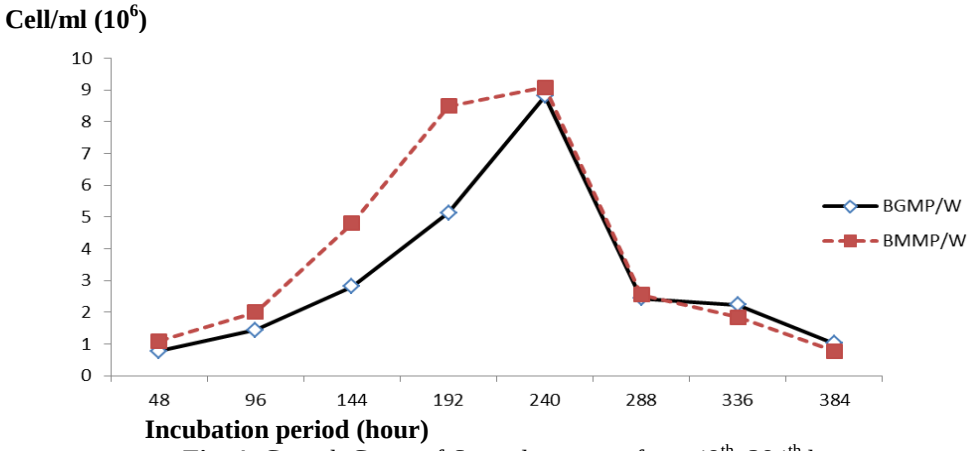

Fig. 4: Growth Curve of *Scenedesmus* sp. from 48th–384th hour

Two inorganic growth media use for freshwater microalgae cultivation with varying chemical composition were studied. The isolates used in this present investigation showed variations in their growth pattern. Both BG-11 and BMM were found to enhance the growth of two microalgae at different densities.

The present investigation deals with the evaluation of growth rate (cell concentration, value) of *Chlorella* sp. and *Scenedesmus* sp. which showed variations in their growth pattern in the two growth media. In general, BMM was found to greatly influence the growth of the two microalgae of freshwater bodies than BG-11 medium. *Chlorella* sp. exhibited it maximum growth in Bristol Modified Medium (BMM). The growth of *Chlorella* sp. and *Scenedesmus* sp. in different culture media was evaluated by microscopic cell counting value.

Based on the density concentration measurement, it was observed that at 240th hour, growth of *Chlorella* sp. has been highly favoured by BMM (fig.3). From the 48th hour onwards, the cell concentration value of Chlorella sp. gradually increased and reached maximum value at 240th hour in BMM, but concentration decreased progressively with further increase in time. *Chlorella* sp. attained it growth peak at 288th hour and further decreased with time. In case of *Scenedesmus* sp., the growth rate was fairly influenced by BMM (Fig. 4), whereas BG-11 medium did not support the growth at the same rate.

IV. Discussion

Microalgal cultures of *Chlorella* sp. and *Scenedesmus* sp. are significant for various applications beginning from carbon dioxide sequestration to therapeutics and bio-fuels production; hence it is necessary to find the best medium for cell production.

It was reported that N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn are "essential elements" for the growth of microalgae and these elements are utilized in the form of salts [14 and 15]. The amounts of these elements vary from one growth medium to another.

The source of nitrogen, $NaNO_3$ which is present in higher percentage in Bristol Modified Medium than in Blue- Green Medium is responsible for the protein synthesis, hence it account for microalgal growth. In a like manner, BMM contains both K_2HPO_4 and KH_2PO_4 (phosphate sources) whereas only K_2HPO_4 is present in BG-11 Medium as phosphate source for enhanced algal growth. This agrees with the report by [16], that among other media, the highest number of algal colonies and species were isolated using Bristol Modified Medium. A report by [17], suggested that K_2HPO_4 improved dark reaction in *Selenastrum* sp. that resulted to its rapid growth. $MgSO_4$ was the magnesium source which stimulates the chlorophyll production which in turn increased overall cell yield thus magnesium concentration indirectly affected the growth of the isolate which was similar to the earlier study on *Chlorella* sp. by [7].

In this work, Na_2CO_3 and Na_2SiO_3 were the sources of carbon and silica; higher cell concentration value indicated that these nutrients also supported algal growth. Furthermore, these nutrients are responsible for alkaline buffering in medium. Sharma *et al*. (2011), [18], reported sodium carbonate and silicate in Chu 10 medium was responsible for higher algal growth.

Ferric iron solution in BMM was the source of iron the medium which resulted in higher biomass production when compared to BG-11 medium. [7], noted that iron was one of the vital elements in algal growth which deficiency results to growth retardation.

Trace elements such as Mn, Cu, Zn, Co and B are known to support the algal cell accumulation in small amount but retard cell growth when in excess [19]. The BG-11 medium appeared to contain high concentration of these micronutrients which is responsible for low growth of these freshwater organisms.

V. Conclusions

In this study, effect of Bristol Modified Medium and Blue- Green Medium on the growth of two fresh water microalgae namely *Scenedesmus* sp. and *Chlorella* sp. were investigated. It was clearly observed that BMM has a greater influence on the growth of *Chlorella* sp. and *Scenedesmus* sp. when compared with BG-11 medium. But of two algae, *Chlorell*a sp was favoured by BMM. Molecular characterization of these isolates is currently under investigation.

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