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PRELIMINARY STUDY OF DIFFERENT MEDIAS AND VARIOUS PROCESS PARAMETERS ON THE GROWTH OF BLUE-GREEN ALGAE (*ANABAENA AMBIGUA*)

M.B.VENKATARAMANA REDDY*, S.S.LAKSHAMANA RAO AND C.S.RAO

Department of Biotechnology, Sreenidhi Institute of Science and Technology, (An Autonomous Institution) Ghatkesar,Hyderabad-501301, AndhraPradesh.

ABSTRACT

In this study the fresh water grown Blue-Green Algae (*Anabaena ambigua*) is studied for its maximum growth. This Blue-Green Algae (*Anabaena ambigua*) growth is studied in detail in three different media viz.,Fog's media,BG-11, BBM , there was a significant change observed in growth of the species *Anabaena ambigua* at different light intensities and different media, during 15-days growth. Growth was better in B.G.-11 media at 7000lux light intensity when compared to other media. Biochemical composition(Protein content, carbohydrate content,) of the species *Anabaena ambigua* are estimated. Preliminary studies were done on various parameters like pH,Temperature,light intensity, and agitation on the growth of Blue-Green Algae (*Anabaena ambigua*).

KEYWORDS: **B.G-11 media, *Anabaena ambigua,* Light intensity, pH, Blue-Green Algae, Fog's media**

M.B.VENKATARAMANAREDDY
Department of Biotechnology, Sreenidhi Institute of Science and Technology, (An Autonomous Institution) Ghatkesar,Hyderabad-501301, AndhraPradesh.

INTRODUCTION

Depletion of existing fuels, rapidly growing economy, are increasing the demand for fossil fuels. Their combustion is having adverse effect on environment in the form of pollution[1]. To protect the environment and meet the needs of sufficient energy , more attention is made on alternative energies[2,3]. Energy obtained from non-renewable sources like sun, wind, geothermal energy are not sufficient to meet the demand. So scientists are focussing more on clean and greenfuels[4]. Biodiesel has received considerable attention in recent years as it is biodegradable, renewable and non-toxic fuel[5,6]. It emits less gaseous pollutants than conventional diesel fuel, and can work directly in diesel engines with no required modifications[7,8]. Much of research is done on the oil obtained from plants like soybean, corn, coconut, jatropha , microalgae etc. The most important microalgae in terms of abundance are Diatoms, green algae, golden algae and Blue-Green Algae (prokaryotic microorganisms). Microalgae grows rapidly and doubling time is also less, so more research work is focussed on obtaining biodiesel from microalgae. Blue-Green Algae(Cyanobacteria) has wide applications in pharmaceutical industry[9], food industry[10,11], reducing CO_2 emissions[12,13], as biofertilizers[14] etc. The Blue-Green Algae also constitutes as a source of valuable products such as phycobiliproteins, polysaccharides, protein for feed and food[15,16,17]. So in present study fresh water Blue-Green Algae(*Anabaena ambigua*) is grown in different media with varying light intensities, to check where better biomass is obtained. Protein content in microalgal biomass is estimated[18]. carbohydrate content in microalgal biomass is also estimated[19,20]. Once the better media for growth is identified, The effect of Process parameters like pH, temperature, agitation , light intensity are also varied to study their effect on biomass formation.

MATERIALS AND METHODS

The culture Blue-Green Algae (*Anabaena ambigua*) used in this experiments is obtained from NCIM, Pune with Accession number 2785. The culture grown on solid agar media was subcultured in 150ml Erlenmeyer flasks This culture was grown photoautotrophically in the given media(FOG's medium) at 28°C under light and dark illumination (18:6) which was kept on the orbital shaker at 110 rpm for 12 days. Now sub-culture is available to perform further set of experiments. Three different fresh water medias Bold's Basal media, BG-11 media are identified where good growth is observed..All the chemicals used in preparing the compositions of different medias are of analytical grade purchased from Hi-Media. Distilled water is used in making these media according to their standard composition.

EXPERIMENTAL DESIGN

Now the growth of the Blue-Green Algae(*Anabaena ambigua*) is compared in two other fresh water medias (Bold's Basal media, BG-11 media) along with the FOG's media. Now the sub- culture grown in FOG's media is transferred into four 150ml Erlenmeyer flasks containing FOG's medium. similarly it is inoculated into four 150ml Erlenmeyer flasks containing Bold's Basal medium and BG-11 medium. Experiments were carried out in duplicates at different light intensities.Now after 5 days of growth each flask of different media is taken out and optical density of fully grown culture is measured using spectrophotometer at an absorbance of 540nm. Then the dry weight of each culture in different media was obtained by taking the flask out from the shaker and allowing the culture to settle at the bottom so that the media is removed, then it was centrifuged for 15min at 4000rpm . The pellet obtained by centrifugation is dried in hot air oven maintained at 65°C and the dry weight is

obtained from an electronic balance. Similar procedure is followed for the flasks containing different media after 10 days of growth, 15 days of growth and 18 days of growth. Now a standard correlation is developed to estimate the dry weight of sample by knowing the optical density.Now duplicates of these three different media(FOG's media, Bold's Basal Media, B.G.-11 media) are maintained at different light intensities of 1500lux, 4000lux, 7000lux.Protein content in the cultures grown in different media and different light intensities are estimated by Lowry's method.Carbohydrate content in the cultures grown in different media and different light intensities are estimated by phenol-sulphuric acid method. The media and Light Intensity where good growth is observed is identified and individual components of that media are studied futher to know which components play key role in the growth of the species *Anabaena ambigua*. Even process parameters like pH, Light Intensity, agitation, temperature effects are studied .

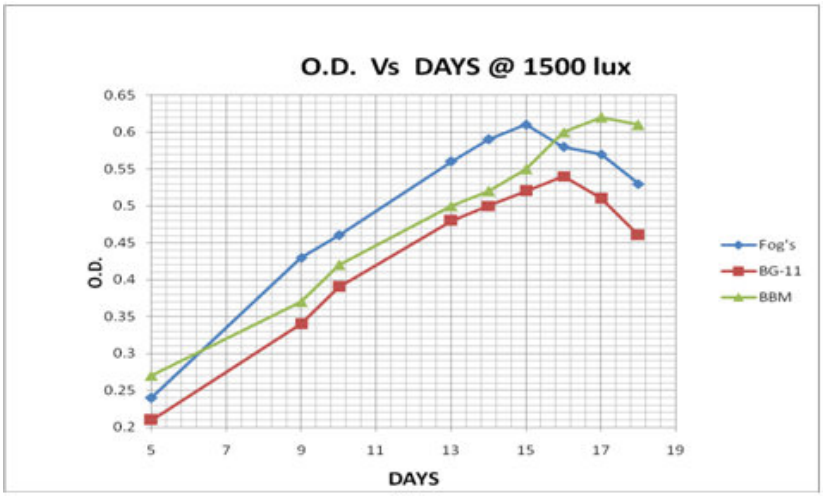
RESULTS AND DISCUSSION

Growth of sub cultured species (*Anabaena ambigua*) is studied at different light intensities (1500lux, 4000lux, 7000lux) in different media(FOG's, Bold's Basal, BG-11 media).

Case(i): Growth of sub-cultured species (*Anabaena ambigua*) in three different medias at 1500lux is observed for 18days and optical densities were recorded periodically. A graph is plotted between days of growth and optical density.

Graph 1

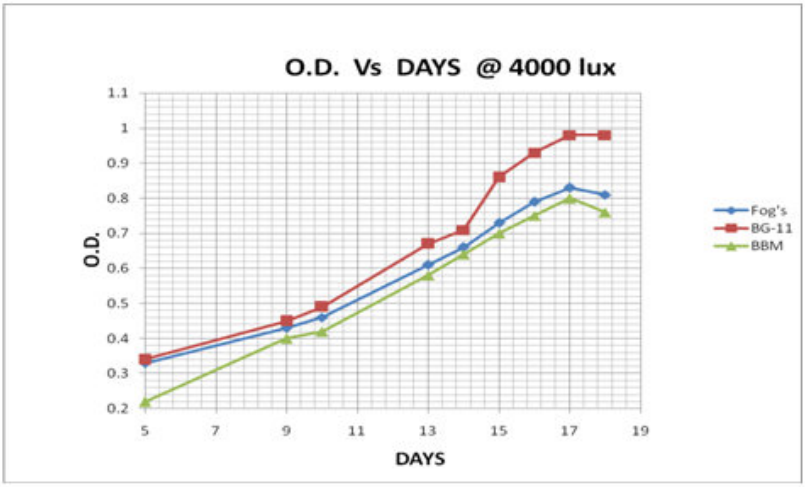
Optical density vs Days at light intensity of 1500lux



As shown in graph 1, there were significant differences on the growth of cells beginning from 9 days of cultivation period in different culture media at the light intensity of 1500lux.The cell growth reached the maximum in BBM and FOG'S culture mediums on the 15th and 18th day respectively of cultivation and then growth in FOG'S and BG-11 medium start to decrease slightly.

Case(ii): Growth of sub cultured species (*Anabaena ambigua*) in three different medias(Bold's,BG-11,FOG's) at 4000lux is observed for 18days and optical densities were recorded periodically. A graph is plotted between days of growth and optical density.

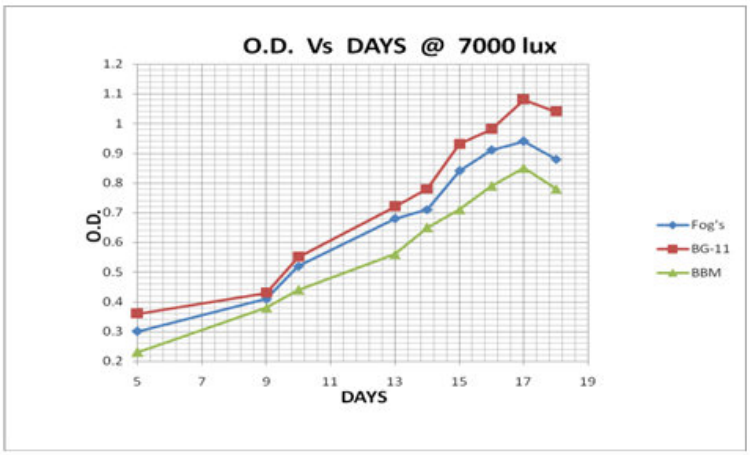
Graph 2
Optical density vs Days at light intensity of 4000lux



As shown in graph 2, there were significant differences on the growth of cells beginning from 7 days of cultivation period in different culture media at the light intensity of 4000 lux.The cell growth was almost same in BG-11 media and Fog's media upto 9 days later the growth was observed to be rapid in BG-11 media than compared to Fog's media and BBM media.

Case(iii): Growth of sub cultured species (*Anabaena ambigua*) in three different medias (Bold's,BG-11,FOG's) at 7000lux is observed for 18days and optical densities were recorded periodically. A graph is plotted between days of growth and optical density.

Graph 3
Optical density vs Days at light intensity of 7000lux



As shown in the graph 3 there were significant differences in growth of cells beginning from 10th day of cultivation period in different culture media at light intensity of 7000lux.Better growth was observed in BG-11 media when compared to Fog's and BBM media. However the overall growth increases at 7000 lux light intensity in all the medias when compared to the growth at 4000lux . Further increase in light intensity decreases slightly the overall growth rate in all media and one of the reason for this could be the photoinhibition effect. The Specific growth rates of different media(Bold's,BG-11,FOG's) at different light intensities is tabulated as follows

Table 1
Specific growth rates and doubling times at different light intensities

Culture Media	Light Intensity(lux)	Initial Conc(g/l)	Final Conc(g/l)	Sp Growth ($days^{-1}$)	Doubling Time(hr^{-1})
Fog's	7000	0.0045	0.1804	0.2839	58.584
BG-11	7000	0.0045	0.1932	0.2892	57.510
BBM	7000	0.0045	0.1712	0.2799	59.421
Fog's	4000	0.0045	0.1726	0.2805	59.294
BG-11	4000	0.0045	0.1889	0.2851	58.012
BBM	4000	0.0045	0.1523	0.2709	61.395
Fog's	1500	0.0045	0.0435	0.1745	95.312
BG-11	1500	0.0045	0.0248	0.1312	126.728
BBM	1500	0.0045	0.0257	0.134	124.119

As the light intensities increased from 1500 lux to 7000lux the specific growth rate increased(Doubling time decreased) for all media and when increased from 4000lux to 7000 lux the specific growth rate slightly increased (Doubling time slightly decreased).From the above table 1 we can draw a conclusion that the specific growth rate of BG-11 media at 7000 lux is higher(Doubling time is lower) when compared to other media and light intensities.

Table 2
Protein composition at 7000lux

Media	O.D.	Protein Concentration
FOG's	1.42	24%
BG-11	1.56	27.2%
BBM	1.10	20%

Table 3
Carbohydrate composition at 7000lux

Media	O.D.	Carbohydrate Concentration
FOG's	1.56	21.8%
BG-11	1.48	20.1%
BOLD's	1.39	18.3%

Table 4
Protein composition at 4000lux

Media	O.D	Protein Concentration
FOG's	1.38	23.1%
BG-11	1.57	27%
BBM	1.12	21%

Table 5
Carbohydrate Composition at 4000 lux

Media	O.D	Carbohydrate Concentration
FOG's	1.5	19.1%
BG-11	1.46	20%
BBM	1.37	19%

Table 6
Protein Composition at 1500 lux

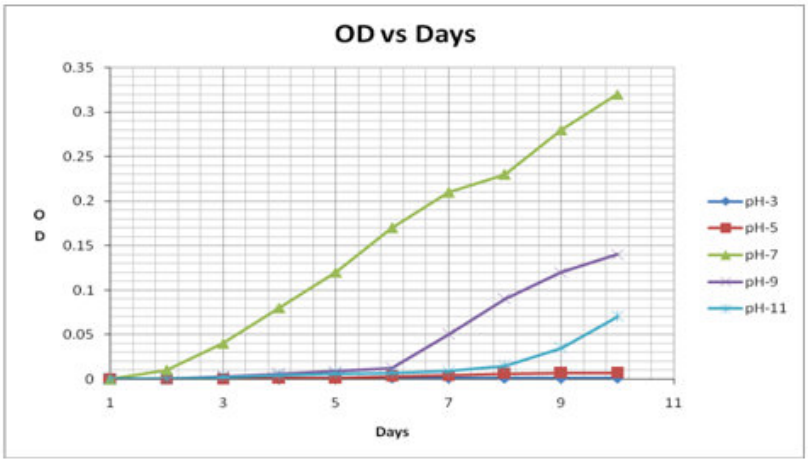
Media	O.D	Protein Concentration
FOG's	0.97	19%
BG-11	1.2	21%
BBM	0.98	18%

Table 7
Carbohydrate Composition at 1500 lux

Media	O.D	Carbohydrate Concentration
FOG's	1.2	16%
BG-11	1.18	18%
BBM	1.12	17.2%

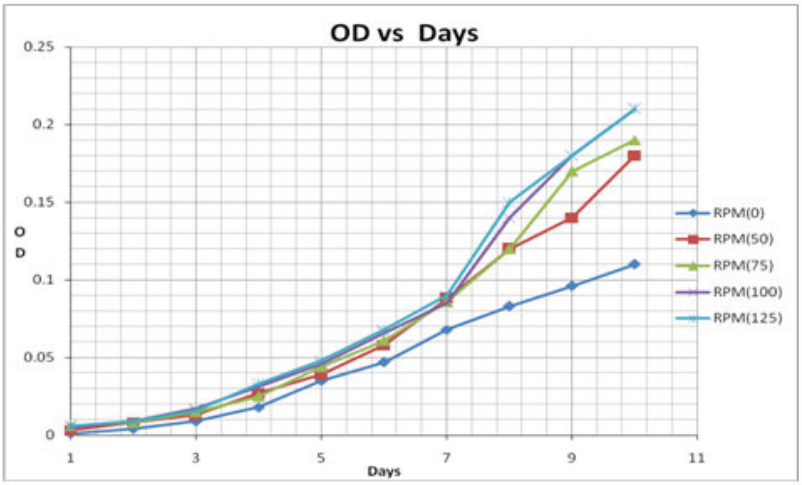
Protein and carbohydrate concentration increases from 1500lux to 4000lux. No significant changes in protein and carbohydrate concentration is observed from 4000lux to 7000 lux lightintensity. Now from the above experiments it is observed that B.G.-11 media at 7000lux light intensity is better for the growth of Blue-Green Algae (*Anabaena ambigua*). The effect of Process parameters like pH, Temperature, agitation, Light intensity were studied on the sub-cultured (*Anabaena ambigua*) in B.G.-11 media. Different pH (viz.,3,5,7,9,11) are maintained in the culture media and growth is observed as follows.

Graph 4
Effect of pH on culture growth



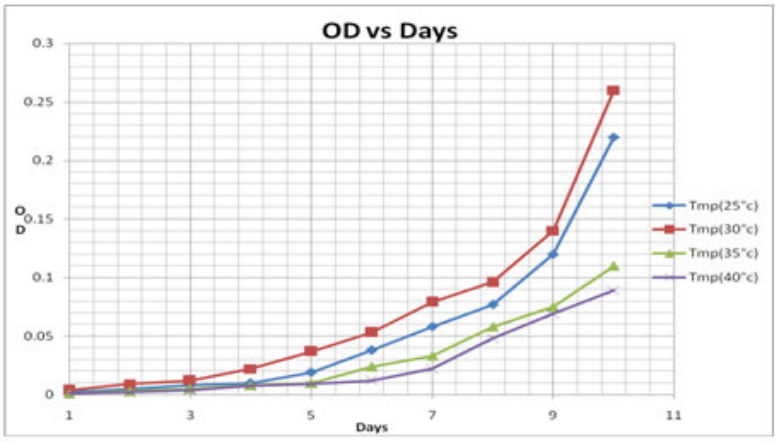
From the graph 4 it is observed that better growth is observed at pH-7, than followed by pH-9.No growth is observed at pH-3 and 5.Little growth is observed at pH-11. Different R.P.M's like 0,50, 75, 100, 125 are maintained in the culture media and growth is observed as follows.

Graph 5
Effect of R.P.M on culture growth



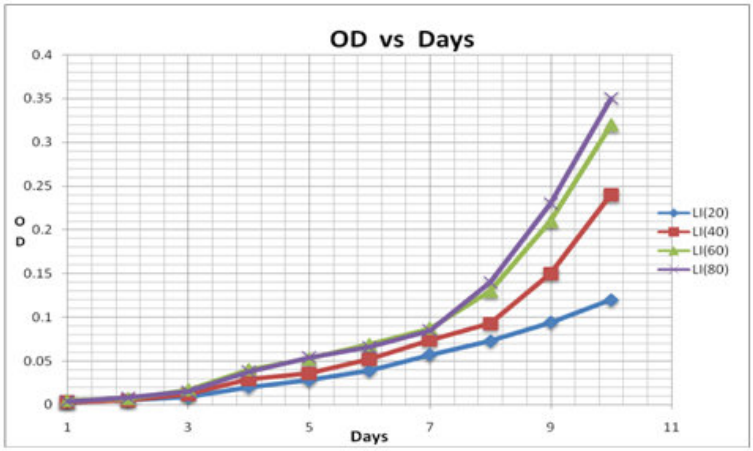
From the above graph 5 it is observed that as the R.P.M increases growth increases. No much difference is observed in the growth as R.P.M increases from 100 to 125.Effect of different temperatures like 25^0 c, 30^0 c, 35^0 c, 40^0 c are studied on culture growth.

Graph 6
Effect of Temperature on culture growth



From the above graph 6 it is observed that as the temperature increases from 25^0 c to 30^0 c the growth increases.As the temperature increases further the growth decreases.Different Light intensities varying from 20 µmol/m^2s to 80 µmol/m^2s are studied on culture growth

Graph 7
Effect of Light Intensity on culture growth



From the above graph 7 it is observed that as the light intensity increases from 20 µmol/m^2s to 80 µmol/m^2s . Further increase in light intensity . slightly decreases the growth rate and one of the reason could be photoinhibition effect.

CONCLUSION

Among different medias studied, good growth of the Blue-Green Algae(*Anabaena ambigua*) was observed in BG-11 media. Preliminary tests were done on the effect of parameters like pH, Temperature, agitation, and Light intensity on the growth of the species. The above said parameters were studied by classical method(Changing one independent variable keeping all other parameters at constant value.) which gives a broad idea of the effect of these variables. However a detailed study of the constituents of BG-11 media and process parameters(pH, temperature, agitation, light intensity) can be done at a time by using statistical technique like Plackett-Burman design and the variables that play key role in the growth of the species are screened out and further optimization of these variables can be done to find where maximum biomass is obtained.

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