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Comparitive Studies of Different Medias and Various Process Parameters on the Growth of Anabaena Ambigua

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ABSTRACT: Present study focuses on growth of blue-green algae (*Anabaena ambigua*) in fresh water with different media and light intensities. The growth was observed in three media viz., BBM, Fog's and BG-11 at various light intensities viz., 1500 lux, 4000 lux and 7000 lux. Better growth was observed in BG-11 media at 7000 lux light intensity with specific growth rate of 0.289 day^{-1} compared with other combinations. Preliminary studies were carried out by OFAT method (One Factor At a Time) using BG-11 media at 7000 lux and was observed that maximum growth was at pH of 7, RPM of 100, temperature of 30°C .

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

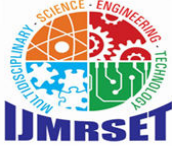
I. INTRODUCTION

Depletion of existing fuels, rapid growth of economy, are increasing the demand for fossil fuels. Their combustion has adverse effect on environment in the form of pollution¹. To protect the environment and meet the needs of sufficient energy, more attention has to be 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 greenfuels⁴. 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 industry⁹, food industry^{10,11}, reducing CO₂ emissions^{12,13}, as biofertilizers¹⁴ etc. The blue-green algae also constitutes as a source of valuable products such as phycobiliproteins, polysaccharides, protein for feed and food¹⁵. So in present study fresh water blue-green algae (*Anabaena ambigua*) was grown in different media with varying light intensities, to check where better biomass is obtained. Once the better media for growth was identified, the effect of different process parameters like pH, temperature, agitation, light intensity were studied on biomass formation.

II. MATERIALS AND METHODS

The culture *Anabaena ambigua* used in these experiments was obtained from NCIM, Pune with accession number 2785. The culture grown on solid agar media was subcultured in 150 ml 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 in 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 viz., Bold's Basal media, BG-11 and Fog's media were identified to test the growth of the culture. All the chemicals used in preparation of different medias are of analytical grade and were purchased from Hi-Media. Distilled water was used in making these media according to their standard composition.



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III. EXPERIMENTAL DESIGN

The growth of the *Anabaena ambigua* was compared with other fresh water media viz., Bold's Basal media, BG-11 media along with the Fog's media. The sub-culture grown in FOG's media was transferred into 150 ml Erlenmeyer flasks containing FOG's medium, Bold's Basal medium and BG-11 medium. Experiments were carried out in duplicates at different light intensities.

After 5 days of growth each flask of different media was taken out and optical density of fully grown culture was measured using spectrophotometer at an absorbance of 540 nm. 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 15 min at 4000 rpm. The pellet obtained by centrifugation was dried in hot air oven maintained at 65 °C and the dry weight was 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. A standard correlation is developed to estimate the dry weight of sample by knowing the optical density.

Now duplicates of these three different media viz., Fog's media, Bold's basal media, BG-11 media were maintained at different light intensities of 1500 lux, 4000 lux, 7000 lux.

IV. RESULTS AND DISCUSSIONS

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

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

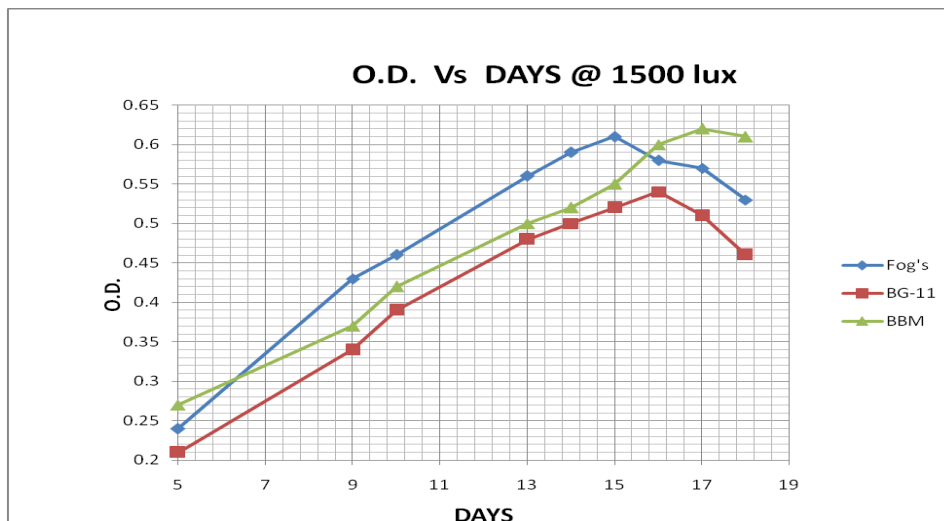
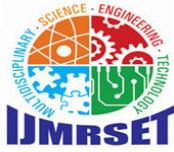


Fig1: Optical density vs Days at light intensity of 1500 lux

In Fig 1, there were significant differences observed during the growth of cells beginning from 9 days of cultivation period in different culture media at the light intensity of 1500 lux.

The cell growth reached maximum in BBM and Fog's culture mediums on 15th and 18th day respectively of cultivation and then growth in Fog's and BG-11 medium started to decrease slightly.



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

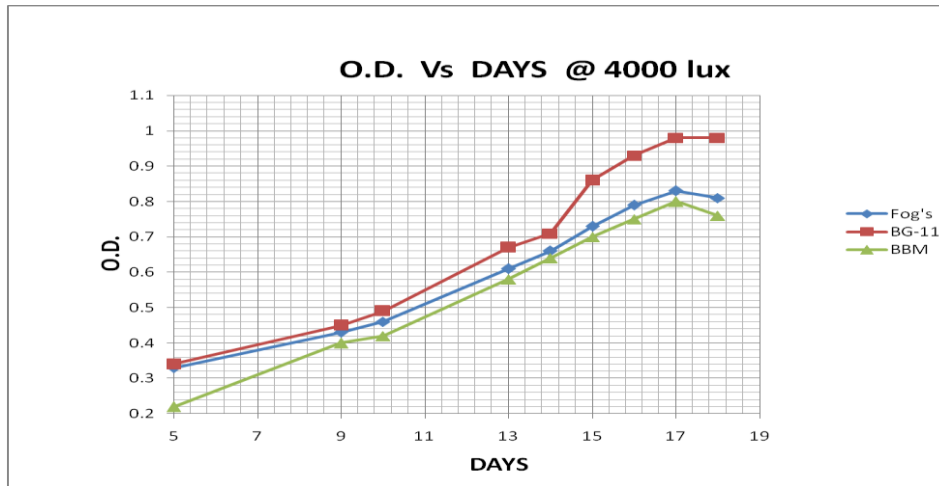


Fig 2: Optical density vs Days at light intensity of 4000 lux

In Fig 2, there were significant differences during 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 7000 lux was observed for 18 days and optical densities were recorded periodically. A graph was plotted between days of growth and optical density.

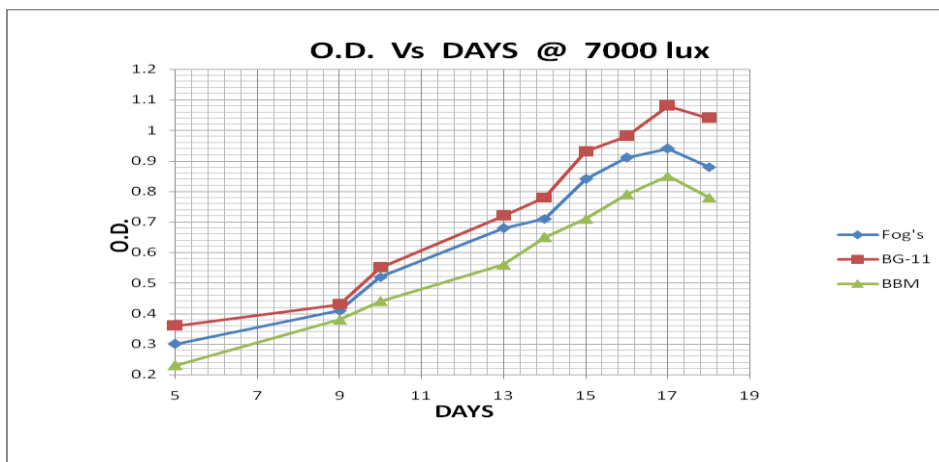


Fig 3: Optical density vs Days at light intensity of 7000 lux

In Fig 3 significant differences in growth of cells starting from 10th day of cultivation period in different culture media at light intensity of 7000 lux.



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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 with the growth at 4000 lux. Further the increase in light intensity decreased 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 were tabulated as follows:

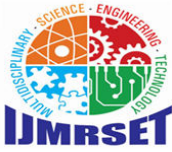
Table 1 Specific growth rates and doubling times at different light intensities

| 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 7000 lux the specific growth rate increased (Doubling time decreased) irrespective of media and when increased from 4000 lux to 7000 lux the specific growth rate slightly increased (Doubling time slightly decreased). From the above table 1 we can conclude that the specific growth rate of BG-11 media at 7000 lux is higher(Doubling time is lower) when compared with other media and light intensities.

From the above experiments it was observed that BG-11 media at 7000 lux light intensity was better for the growth of Anabaena ambigua. The effect of the process parameters like pH, temperature, agitation and light intensity were studied on the sub-cultured Anabaena ambigua using BG-11 media.

Different pH(viz.,3,5,7,9,11) were maintained in the culture media and growth was observed as follows.



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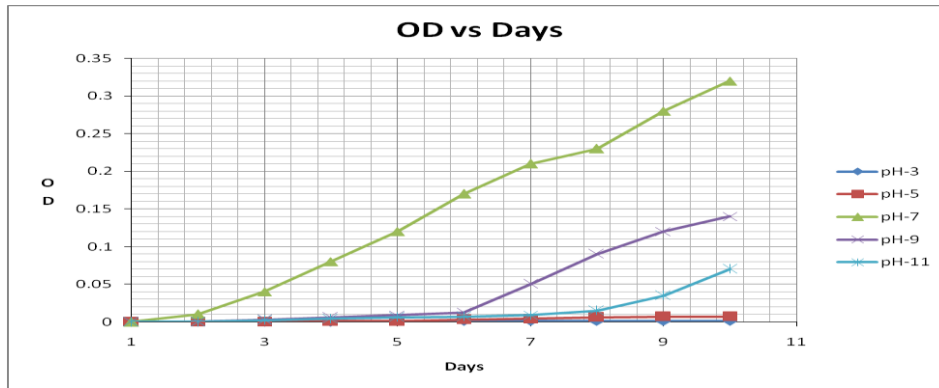


Fig 4: Effect of pH on culture growth

From the Fig 4 it was observed that better growth is at pH-7, followed by pH-9. No growth was observed at pH-3 and 5. Little growth was observed at pH-11.

Different R.P.M's like 0,50, 75, 100, 125 were maintained in the culture media and growth was observed as follows.

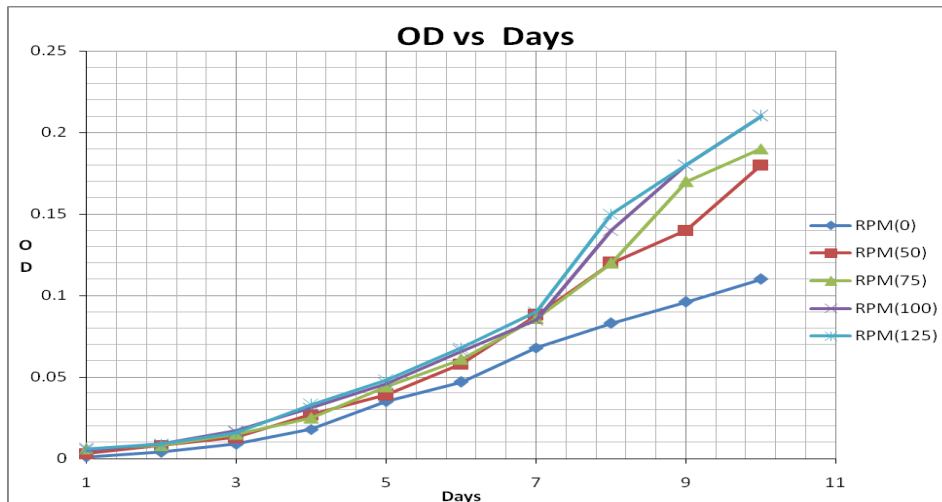


Fig 5: Effect of R.P.M on culture growth

From Fig 5 it was observed that as the R.P.M increases growth increases. No much difference was observed in the growth as R.P.M increases from 100 to 125.



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Similarly effect of different temperatures like 25 °c, 30 °c, 35 °c, 40 °c was studied on culture growth.

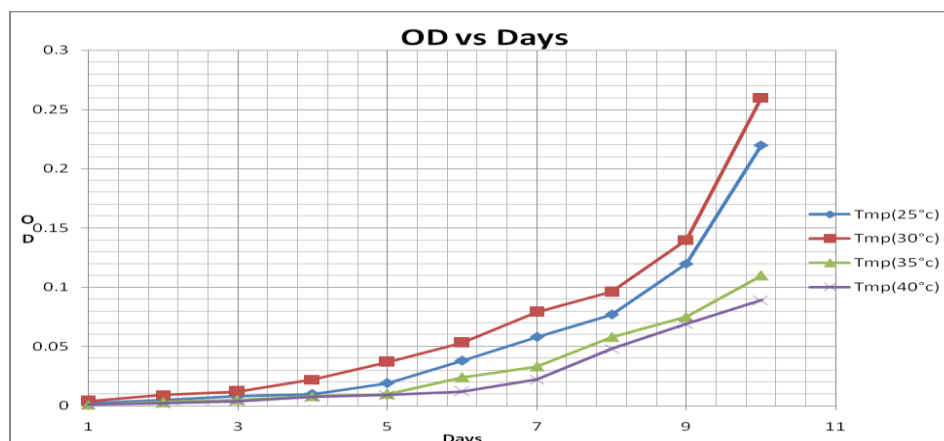


Fig 6: Effect of temperature on culture growth

From Fig 6 it was observed that as the temperature increased from 25 °c to 30 °c the growth increased. As the temperature increased further, the growth decreased.

V. CONCLUSIONS

Among different medias studied, good growth of *Anabaena ambigua* was observed in BG-11 media. Preliminary tests were done on the effect of parameters like pH, temperature, and agitation on the growth of the species. The above said parameters were studied by OFAT method (Changing one independent variable keeping all other parameters at constant value.) which gives a broad idea of the effect of these variables.

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