



STUDY OF GROWTH PARAMETERS ON MEMBERS OF CHLOROCOCCALES

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ABSTRACT

Algae are become one of the most auspicious long-term sources for food, oil, fuel and other co-products. Algae produced around 15,000 novel compounds and they were resolved through chemically. Most of the microalgae generated significant products such as fatty acids, antioxidants, polymers, sterols, carotenoids, and enzymes. The aim of the study is the growth parameter analysis, especially growth media, pH and temperature. These three parameters are major role for cultivation of micro algae. Different types of medium were used BBM, M-8 and BG11 for *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibrainum*, different pH such as 4, 4.5, 5 up to 8.5 with 0.5 interval gap and different temperatures such as 20°C to 40°C with the interval of 5°C. BBM showed significant growth with 212, 262, and 342 mg/L/day, pH 7.5, 7.5 and 6.5 with 258, 293 and 432 mg/L/day and 30°C with 192, 255, 316 mg/L/day.

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INTRODUCTION

Microalgae are simple plants which range from micro to macro in size and unicellular to multicellular in cell organization. They have the capability to utilize the atmospheric CO₂ and they can observe solar energy greater than plants (10-50 times). The higher growth rates of algae have resulted in the higher biomass production than the oil crops (Wang *et al.*, 2008). This makes the algae biomass as the best source for oils for fuel production and other utilities like food, feed, fodder, etc. Microalgae are considered as the better choice for biofuel producing source, because it doesn't compete with the food, and do not require much land for growth, don't cause deforestation and GHG (Abomohra *et al.* 2016).

Major significant possessions of algae such as Grow fast, have high biofuel yields, consume CO₂, biomass used for feed, fuel and food, and it can be grown even in the Sea. In the part of aquaculture field, micro algae especially microphytes are best feed for altering bivalves. The symbiotic relationship was formed between host organisms with both of chemosynthetic and photosynthetic which supply then polyunsaturated fatty acids and vitamins. These properties were best feed for the bivalves of its growth and development.

When compare to other energy crops, micro algae contained several advantages mainly higher photosynthetic competence as well as development rates and biomass production. Most of the micro algae cultures are to have featured to accumulate

lipids in their cell, while it was perceived that the higher lipid accumulation occurs during the condition when the nitrogen source is limited (Reiten *et al.*, 1994). *Scenedesmus dimorphus* is one of the coenobium forming unicellular freshwater green algae in class of *Chlorophyceae*. The name explains as "having two forms". The colonial green algal genus *Coelastrum* has 25 species (Venkataraman and Goyal 1962). All the species confined polyhedral, polygonal or hollow spherical coenobium and the cells in a coenobium vary from 4 to 32, depending upon the culture conditions (Wile 1909, Rayss 1915). An individual cell of the *coenobium* is joining with each other by surface pads of mucilage while in others they are joined by processes, polar discs or papillae on the wall in the other species (Kwong 1975 and Fritsch 1945). The *Selenastrum bibrainum* cells are fusiform, narrow, semilunate to fusiform, gradually pointed ends, arcuate. Planktonic colonies sometimes solitary, colonies are 2 to 8 celled, mostly the colonies arranged with the convex side at towards the centre of the colony (Silva *et al.* 2017).

The biodiversity of microalgae is enormous and they represent an almost untapped resource. Around 200,000 to 800,000 species in many different genera exist and among this, described around 50,000 species (Cardozo *et al.*, 2006) and from the algal biomass, 15,000 of novel bioactive compounds originated. As per 2012 200,000 species has been identified (Koller *et al.*, 2012) and many yet to be identified, which shows the highest diversity of microalgae. These microalgae can be used for various applications including the recent emerged and highly focused field such green energy (Stengel *et al.*, 2011).

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METHODOLOGY

Algae Strain

Scenedesmus dimorphus, *Coelastrum microporum* and *Selenastrum bibrainum* were cultures were confirmed through compound microscope. The target algae were cultured by transferring to sterilized test tubes which were contained BBM media. The fresh cultures were confirmed by through compound microscope. All the cultures were maintained the temperature 28°C to 30°C until the medium turns green. Before any contamination occurred, all the cultures were performed fresh culture by the followed fresh culture protocols. Then, the fresh cultures were involved to biomass production used sterilized Duran bottle 2L (batch PBR) which were contained 900mL of medium.

Growth medium parameters

According to Ali husein *et al.*, 2103, the different type's growth media were selected. Bold's Basal Medium (BBM) was prepared with the help of distilled water (Bischoff and Bold 1963; Rowley, 2010). BG11 medium and M8 medium were prepared by Mandalam and Palsson, 1988 ; Guillard and Ryther, 1962 protocols with small modifications. All the chemicals used were of analytical grade unless pointed out clearly. Three different cultures were cultivated by three different culture media. Each culture was cultivated by each three different media. The cultures were maintained at 28°C to 30°C and in triplicates.

pH measurement

In this study, different pH such as 4, 4.5, 5 up to 8.5 with 0.5 interval gap was maintained to the culture media. After growth media parameters were done, the culture media was selected. The growth was estimated by monitoring the optical density at 670 nm using UV-vis spectrophotometer. The dry weight was calculated to establish biomass concentration and pH measurement were made used standard methods (APHA, 1998). At every twenty four hours, pH measurements were made in triplicate method. All the biomass cultures were maintained at 28°C to 30°C. The pH adjustments were done by using of 5N Hydrochloric acid and 5N Sodium Hydroxide.

Temperature parameter

Three different biomass cultures were used to analysis of suitable temperature conditions. Temperatures were maintained from 20°C to 40°C with the interval of 5°C. The cultures were maintained shaker incubator. The growth was monitored at every 12 hours with help of UV-vis spectrophotometer at 670nm. This study was performed triplicate method.

Statistical analysis

Overall studies such as Growth medium parameters, pH measurement and Temperature parameter were performed triplicate method so used average data calculated by Microsoft Excel version of 2013 and the graph was done by Origin 8.

RESULTS AND DISCUSSIONS

Scenedesmus dimorphus, *Coelastrum microporum* and *Selenastrum bibrainum* are one of the significant micro algae. Generally, microalgae which is a promising alternative feedstock for the future generation of bio-fuels, because they have significant properties such as relatively high lipid

content, proficient growth rate through development on non-arable land and stabilized water, and it can be harvested daily with no seasonal limitations (Gouveia and Oliveira, 2009). Microalgal had best properties in biofuel production at high level in commercial ways and it can able to produce methane in an anaerobic digestion at biomass production (Spolaore *et al.*, 2006), biodiesel production derived from an oil (Banerjee *et al.*, 2002; Gavrilesco and Chisti, 2005), biohydrogen and bioethanol produced by photo biologically (Fedorov *et al.*, 2005; Kapdan and Kargi, 2006).

Cultivation of Algae with different culture medium

Commonly, BBM was widely used for cultivation of algae. Here, three different media was used for the cultivation with small modifications in the formulations. Along with BBM, M-8 and BG11 were used for cultivation of *Scenedesmus dimorphus* (Figure.1), *Coelastrum microporum* (Figure.2) and *Selenastrum bibrainum* (Figure.3). These cultures were performed biomass production under the condition of normal temperature (28°C to 30°C). Overall 900 mL of media such as BBM, M-8 and BG11 were prepared using of deionised water in 2L buran bottle. Every 12 hours interval, OD values were measured at 670nm by UV Vis Spectrophotometer. *Scenedesmus dimorphus* weight was 212 mg/L/day in BBM, 31 mg/L/day in M-8, 143 mg/L/day in BG11; *Coelastrum microporum* weight was 262 mg/L/day in BBM, 88 mg/L/day in M-8, 198 mg/L/day in BG11; *Selenastrum bibrainum* weight was 342 mg/L/day in BBM, 98 mg/L/day in M-8, 213 mg/L/day in BG11. When compared to M-8 and BG11 media, BBM (Table.1) was showed significant result.

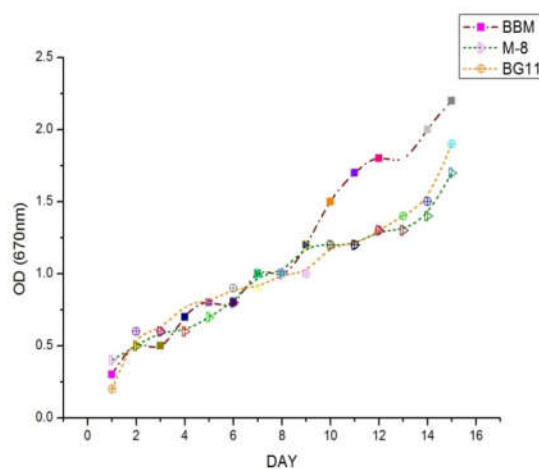


Figure 1 Different medium used for cultivation of *Scenedesmus dimorphus*

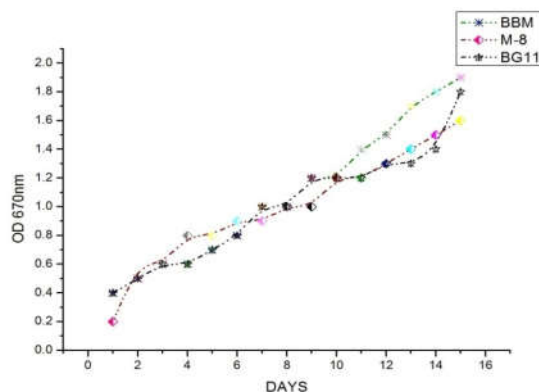


Figure.2. Different medium used for cultivation of *Coelastrum microporum*

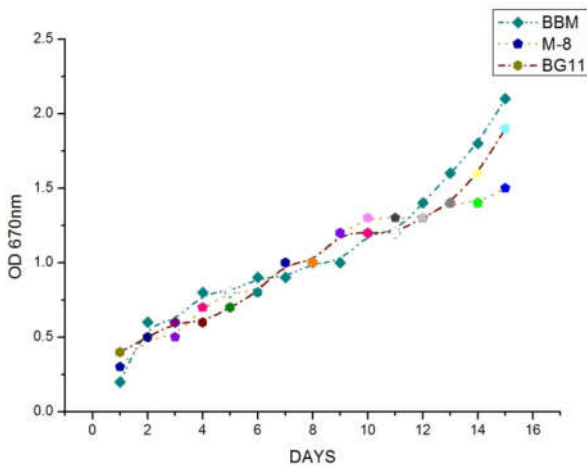


Figure 3 Different medium used for cultivation of *Selenastrum bibraianum*

Table 1 Media selection for *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* (mg/L/day)

Medium	<i>Scenedesmus dimorphus</i>	<i>Coelastrum microporum</i>	<i>Selenastrum bibraianum</i>
BBM	212	262	342
M-8	31	88	98
GB11	143	198	213

Study on pH measurement

Scenedesmus dimorphus, *Coelastrum microporum* and *Selenastrum bibraianum* were performed biomass cultivation used BBM media with different pH parameters 4, 4.5, 5 up to 8.5 with 0.5 interval gap. Every 12 hours interval, OD values were calculated at 670nm by UV Vis Spectrophotometer. *Scenedesmus dimorphus* weight was 12 mg/L/day in pH 4, 14 mg/L/day in pH 4.5, 19 mg/L/day in pH 5, 24 mg/L/day in pH 5.5, 99 mg/L/day in pH 6, 132 mg/L/day in pH 6.5, 198 mg/L/day in pH 7, 258 mg/L/day in pH 7.5, 90 mg/L/day in pH 8, 143 mg/L/day in pH 8.5; *Coelastrum microporum* weight was 11 mg/L/day in pH 4, 15 mg/L/day in pH 4.5, 21 mg/L/day in pH 5, 29 mg/L/day in pH 5.5, 159 mg/L/day in pH 6, 215 mg/L/day in pH 6.5, 212 mg/L/day in pH 7, 293 mg/L/day in pH 7.5, 163 mg/L/day in pH 8, 245 mg/L/day in pH 8.5; *Selenastrum bibraianum* weight was 16 mg/L/day in pH 4, 24 mg/L/day in pH 4.5, 31 mg/L/day in pH 5, 39 mg/L/day in pH 5.5, 360 mg/L/day in pH 6, 432 mg/L/day in pH 6.5, 290 mg/L/day in pH 7, 351 mg/L/day in pH 7.5, 183 mg/L/day in pH 8, 231 mg/L/day in pH 8.5. *Scenedesmus dimorphus* was well grown in pH 7.5, *Coelastrum microporum* was in pH 7.5 and *Selenastrum bibraianum* was in 6.5 (Table.2). Further study of temperature parameters, BBM media was prepared and maintained with suitable pH.

Table 2 Analysis of pH parameter study using of *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* (mg/L/day)

pH	<i>Scenedesmus dimorphus</i>	<i>Coelastrum microporum</i>	<i>Selenastrum bibraianum</i>
4	12	11	16
4.5	14	15	24
5	19	21	31
5.5	24	29	39
6	99	159	360
6.5	132	215	432
7	198	212	290
7.5	258	293	351

8	90	163	183
8.5	143	245	231

Study on Temperature parameters

Scenedesmus dimorphus weight was 21 mg/L/day at 20°C, 35 mg/L/day in 25°C, 192 mg/L/day at 30°C, 132 mg/L/day at 35°C, 73 mg/L/day at 40°C; *Coelastrum microporum* weight was 36 mg/L/day at 20°C, 42 mg/L/day at 25°C, 255 mg/L/day at 30°C, 188 mg/L/day at 35°C; 82 mg/L/day at 40°C; *Selenastrum bibraianum* weight was 46 mg/L/day at 20°C, 160 mg/L/day at 25°C, 316 mg/L/day at 30°C, 246 mg/L/day at 35°C, 92 mg/L/day at 40°C (Table.3). *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* were well grown at 30°C in BBM media with pH 7.5, 7.5 and 6.5.

Table 3 Analysis of Temperature parameter study using of *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* (mg/L/day)

Temperature	<i>Scenedesmus dimorphus</i>	<i>Coelastrum microporum</i>	<i>Selenastrum bibraianum</i>
20	21	36	46
25	35	42	160
30	192	255	316
35	132	188	246
40	73	82	92

CONCLUSION

In hatcheries, microalgae species are synthesized and its application in a different ways to commercial purposes. During the culture of micro algae, studies are estimated the main factors in the success of microalgae in hatchery system as the dimensions of the container/bioreactor with concentration of cells within the reactor and exposure to light/irradiation. BBM, M-8 and BG11 were used for cultivation of *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum*. Among these, BBM showed significant growth cultivation with 212, 262, and 342 mg/L/day. *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* were well grown in pH 7.5, 7.5 and 6.5 with 258, 293 and 432 mg/L/day. In the temperature parameters, *Scenedesmus dimorphus*, *Coelastrum microporum* and *Selenastrum bibraianum* were well grown at 30°C with 192, 255, 316 mg/L/day respectively.

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Author Disclosure Statement

The authors have no conflicts of interest to declare.

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