Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article   **DOI: 10.26479/2022.0805.03**

THE EFFECT OF CULTURE CONDITIONS ON THE GROWTH AND BIOMASS OF MARINE DIATOM *SKELETONEMA COSTATUM*

Rajesh Gandhi Gunti, Prasada Babu Gundala, Paramageetham Chinthala*

Sri Venkateswara University, Dept of Microbiology, Tirupati, Andhra Pradesh, India.

ABSTRACT: Microalgae are promising primary producers involved in sustainable energy. As the oceanic environments are dwindling environments the primary productivity is influenced by light and temperature there by altering the biochemical composition of the algae. *Skeletonema costatum*, being a marine organism can be grown in varied environments however, for higher yield of biomass and lipid production simple modifications in the culture conditions is required. This study helps to exploit this species for mass cultivation to produce biodiesel.

Keywords: Specific growth rate, *Skeletonema costatum*, Phytoplankton, Biodiesel, Exponential phase, Photoperiod.

Article History: Received: Sept 20, 2022; Revised: Oct 12, 2022; Accepted: Oct 26, 2022.

Corresponding Author: Dr. Paramageetham Chinthala* Ph.D.

Sri Venkateswara University, Dept of Microbiology, Tirupati, Andhra Pradesh, India.

Email Address:paramageetham@yahoo.com

1. INTRODUCTION

Rapidly increasing global energy demands and utilization of fossil fuel source and its depletion creates problems such as energy crisis, greenhouse gas emission (NO_2, CO_2 and SO_2), which causes global warming and climatic change problems[1] global warming and fatigue of fossil fuels are major environmental and fuel-efficient issues the world is urgently facing today and due to immortal consumption of fossil fuels and effect of greenhouse-gas emissions on global climate change, the world is urgently compelled to focus on finding possible fuels to the existing fossil fuels[2] so research has begun to focus on alternative biomass-derived fuels[3] researchers and scientists are trying to discover an alternative source for the fuel from renewable sources such as vegetable oil, non-edible oil, algae oil etc. In order to meet the demands, availability of fossil fuels, alternate means of fuels are being investigated. In addition the environmental impact from increased carbon dioxide (CO_2) without balanced CO_2 sequestration has contributed to increases in atmospheric CO_2 levels to suppress the drastically rising above issues scientists showed micro-algae is an alternative source. Microalgae utilized the photosynthesis process in growth system and it can store oils in the form of lipids in their membrane, which is converted into energy source. Among micro-algae, diatoms are the one of the largest primary producers of an aquatic eco-system and it can be considered as a source of high lipid content for the production of bio-fuel. Diatoms have been proposed as the future candidate for bio-fuel production due to number of useful inducing highest photosynthetic efficiency, huge biomass production and highest growth rate having short life span due to their simple structure compared to other energy yielding crops.It is estimated that biomass productivity of diatoms could be 50 times more than that of the switch grass which is the fastest growing terrestrial plants [4] compared to plants, microalgae have encouraged photosynthetic effect and produces high oil content. Diatoms are considered as the most promising substrate for producing biodiesel, Triacylglycerol (TAG) and fatty acids, which are the producers of biodiesel and intern is said to be third generation fuel. Like all other marine organisms, diatom physiology is influenced bylight intensity, salinity, temperature and nutrient concentrations [5] temperature is key factor which manages the algal growth in natural environments [6] it is well known that salinity is an important abiotic factor affecting phytoplankton growth. Open span of temperature and salinity may explain frequent appearance of phytoplankton throughout the year in the ocean [7] nitrogen is easily available and cost effective compared to other factors. In various microalgae, nitrogen plays a vital role in the fatty acids and lipid metabolism [8] cultivation of nitrogen deficient medium could increase the lipid production [9] like other diatoms which produces bio diesel *Skeletonema costatum* also often influence the diatom infinity in coastal waters [10] dried cells are an excellent substrate bed for edible mushroom culture. For copepods, live *Skeletonema* cells form an outstanding feed [11] in the present study, *Skeletonema costatum* was selected due to it is an important coastal organism that can tolerate a wide variety of light regimes and temperatures, and it is an ideal laboratory organism that grows readily in various media. It is also a worst-case selection in terms of Non-toxic bloom management because it grows rapidly and attains high population densities.

Moreover, bio-diesel oil is similar in properties to the standard bio-diesel and is also more stable according to their flash point values.

2. MATERIALS AND METHODS

Sampling site

Marine water samples were collected from the Bay of Bengal at Kottapatnam coastal area, Nellore District, Andhra Pradesh, India at latitudes of 14°09'02.5"N and longitudes 80° 07'39.5"E .(Fig-1).

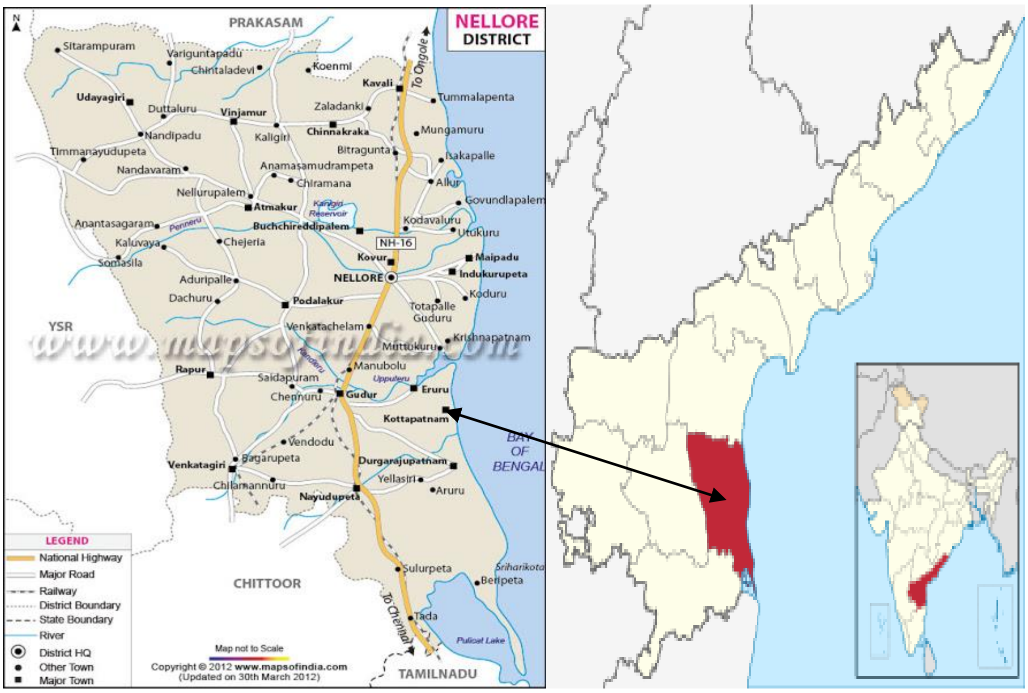

Figure: 1. Map of Nellore district- showing sampling site in Bay of Bengal

Collection and processing of marine water samples

The Marine water samples were collected into a sterile plastic container (25L capacity), capped immediately and processed within 24 hours. This water was filtered with the help of phytoplanktonic mesh (25 µm) and phytoplankton were collected.

Enrichment of diatom Cultures

The collected phytoplankton was inoculated into sterile f/2 media [12] for enrichment. These were incubated at 20 ± 2°C temperature in presence of 2500 lux light intensity with 12hrs dark and 12hrs light cycle for 15 days. The f/2 media helps to grow diatom species alone.

Isolation and Identification of *Skeletonema costatum*.

Based on Morphological studies the *Skeletonema costatum* was isolated from enriched cultured media by using direct microscopic selection method with the help of a fine capillary tube. Pure cultures were obtained after several subcultures on to sterile f/2 agar media containing antibiotics of 200µg Penicillin/100ml and 200µg Streptomycin/100ml *Skeletonema costatum* was observed under the microscope. For identification of *Skeletonema costatum* the algal identification keys were utilized, [13, 14, and 15].

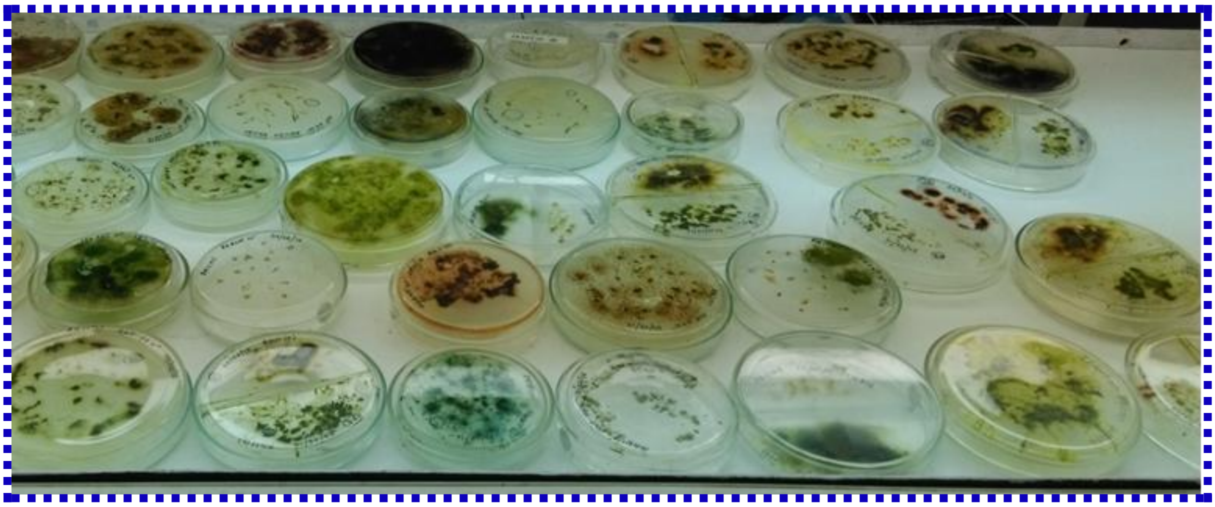

Figure: 2. Petri plates showing pure cultures of *Skeletonema costatum*

Determination of optimum culture condition

The growth was determined at various cultural conditions such as temperature and photoperiod. Various temperatures such as 20ºC, 25ºC, 30ºC and 35ºC and photo period 12h light: 12h dark, 16h light: 08h dark, 20h light: 04h dark were used in f/2 media.

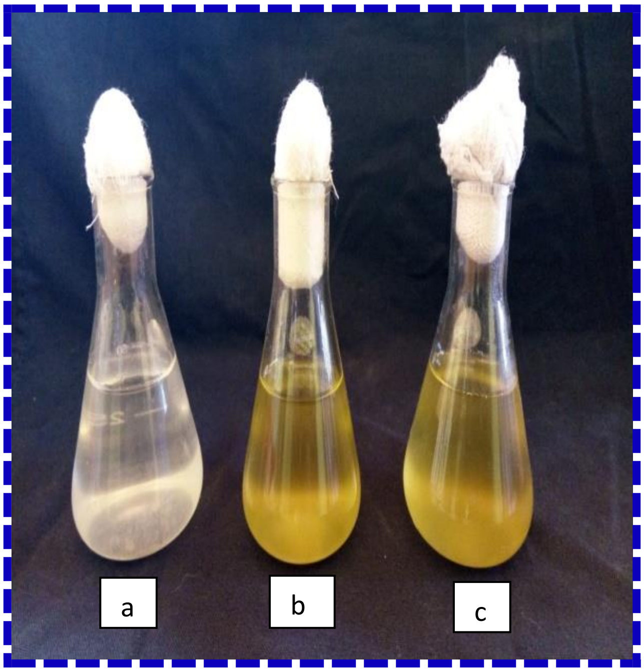

Figure: 3. Effect of light on cultures of *Skeletonema costatum* at different light regime

a. Growth at 20 hrs. Light and 4 hrs dark,
b.Growthat16hrslightand8hrsdark,
c. Growth at 12 hrs light and 12 hrs dark.

Growth kinetics

The growth of the diatom at various temperature and photoperiod was monitored by measuring optical density at 680nm using UV-Visible spectrophotometer (Thermo scientific EVOLUTION- 201) for every 48 hours intervals. Erroneous readings were avoided by brief vortexing of the diatom culture before taking the reading.

Estimation of Biomass

Diatom biomass was estimated for every five days by Richmond method [16]100ml of culture suspension was centrifuged at 2500rpm for 10minutes and pellet was collected. To remove the excess of salts the pellet was washed with de-ionized water and filtered with the help of whatman No.1 filter paper. The diatom cell biomass was allowed for drying at 60°C in a hot air oven until constant weight was obtained. The specific growth rate, division time and generation time was calculated by using the following formula.

Specific growth rate; $K' = Ln (N2 / N1) / (t2 - t_1)$

Divisions per day; $Div.day^{-1} = K' / L2$

Generation time; $Gen't = 1 / Div.day^{-1}$

Where N1 and N2 = biomass at time 1 (t_1) and time 2 (t_2) respectively.

Table-1: Specific growth rate, division rate and generation time of *Skeletonema costatum* at different time intervals

No. of days	Specific growth rate (K’)	Div. day^{-1}	Gen’t(d)
0	0.095	0.137	7.299
05	0.147	0.212	4.716
10	0.184	0.265	3.773
15	0.306	0.441	2.267
20	0.186	0.268	3.731
25	0.167	0.240	4.166
30	0.122	0.176	5.681

3. RESULTS AND DISCUSSION

Skeletonema costatum was selectively isolated from the marine water of Bay of Bengal near Kottapatnam, Nellore District, India. For routine culturing of the diatom *Skeletonema costatum* f/2 media was used. The pure cultures were obtained on solid f/2 media containing antibiotics (Penicillin 200µg/ml and Streptomycin-200µg/ml) and repeated sub culturing (Fig-2&3). Based on the growth, colony morphology, cultural characteristics and microscopic characteristics and on the arrangement of raphe division the diatom *Skeletonema* was identified as *Skeletonema costatum* as per the systematic keys

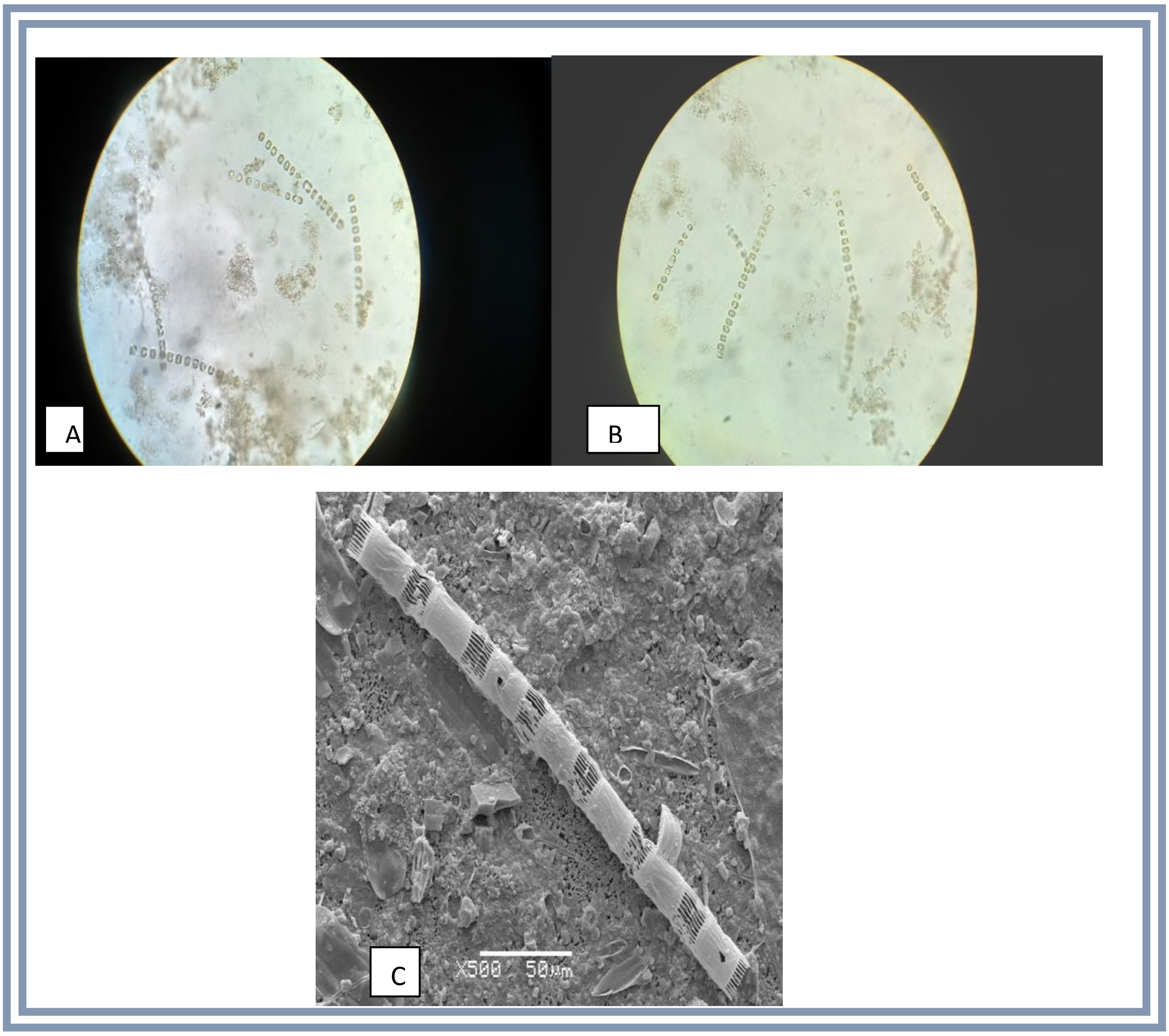

Figure: 4. Images of *Skeletonema costatum* at different magnification
A, B at 40X C – SEM Image

Description of the species

Cells were cylindrical with rounded ends. Cells form long straight chains, held together by fine marginal processes, parallel with longitudinal axis. Spines are straight and slender and unite with the spines of the next cell to form a junction and, two chromophores per cell are present. Nucleus is central. Chain in girdle view, uppermost cell with two chloroplasts was observed (note that the linking structures may be much shorter and less distinct).The *Skeletonema costatum*, valves small, lens shaped with rounded ends and form long and slender chains with the help of marginal spines.

Systematic position of Skeletonema costatum (Greville) Cleve, 1873

Empire	:	Eukaryota
Kingdom	:	Chromista
Division	:	Ochrophyta
Class	:	Mediophyceae
Order	:	Thalassiosirales
Family	:	Skeletonemaceae
Genus	:	Skeletonema
Species	:	*costatum*,

The effect of photoperiod on growth was determined by using the f/2 medium. The photoperiod included 12hours light: 12hours dark, 16hours light: 08hours dark, 20 hours light: 04 hours dark. In 16 hours light : 08hours dark photoperiod optimum growth achieved at 15th day and in 20 hours light and 04 hours dark optimum growth achieved at 10th day (Fig.3&5) However, the maximum optimum growth was recorded at 16hours light: 08hours dark, thus the optimum photoperiod for *Skeletonema costatum* was 16hours light and 08 hours dark. Similarly, the effect of temperature on the growth of *Skeletonema costatum*was also monitored. The temperatures were tested at 20^{o}C, 25^{o}C 30^{o}C and 35^{o}C. The maximum growth was achieved at 15th day (Fig.6) However, the maximum optimum growth was recorded at 20^{o}c. Therefore, the temperature optima for *Skeletonema costatum* is at 20^{o}c. At specified optimum temperature (20^{o}c) and optimum photo period (16: 08hour) growth curve was constructed using f/2 media. The constructed growth curve of *Skeletonema costatum* showed log phase, exponential phase, stationary phase and lag phase. Log phase lasted for 3rdday, exponential phase lasted from 6th day to 10th day, stationary phase 11th to 14th day and lag phase started from 16th to 30th day (Fig-8) the optimum growth was achieved at 14th day. The dry biomass at optimum culture conditions was measured for every five days interval up to 30 days. The maximum biomass was measured at 20th day (0.53g/l) from 21thday onwards biomass was decreased (Fig-7) Based on biomass data specific growth rate, division rate, generation time was calculated and presented in (Table-1) Like all other organisms culture conditions influences the growth of diatom[17] among all the culture conditions temperature and photoperiods are important factors that control the algal growth in natural conditions as well as culture conditions[18,19] reported that diatom growth is influenced by temperature and light, similarly in our studies also physical stimuli like temperature and photo period influenced the growth. In the study diatom that grows at different photo periods should remarkable changes in growth as reported by [20] temperature is very crucial factor for the growth and physiology of micro algae because it directly induces the photosynthesis and biochemical composition and other physical process. In our study optimum temperature is 20°C ± 2°C was observed on 15th day for similarly to [21] also found that temperature is also crucial factor to influence the growth of *Nanochloropsis*. Thus optimum culture condition for *Skeletonemacostatum* is 20°C ± 2°C temperate and 16L:08D on 15th day at 2500lux photo period and this can also applied for mass cultivation *in vitro* system for biodiesel production.

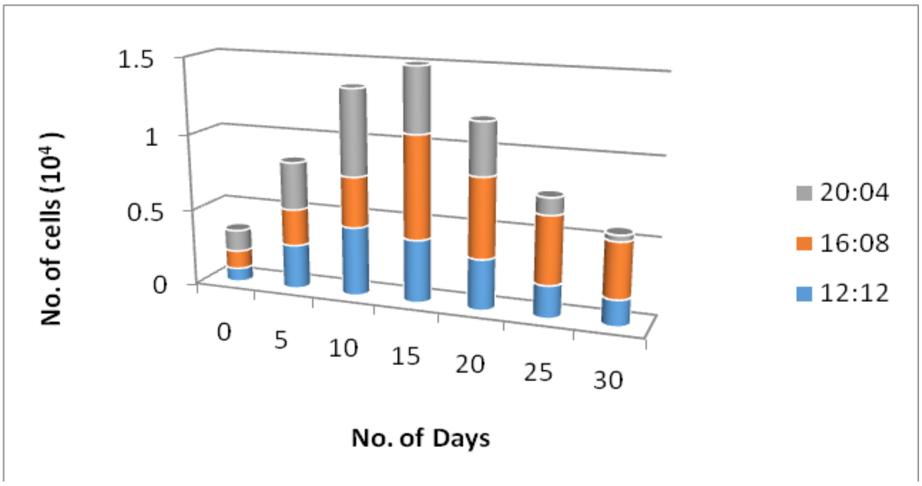

Figure: 5 Effect of light on growth of *Skeletonema costatum* **at different light regimes**

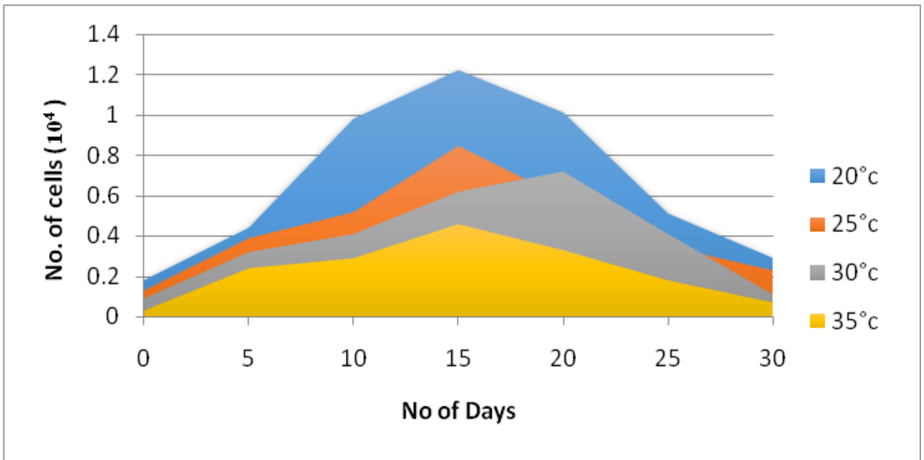

Figure: 6 Effect of growth of *Skeletonema costatum* **at different temperatures**

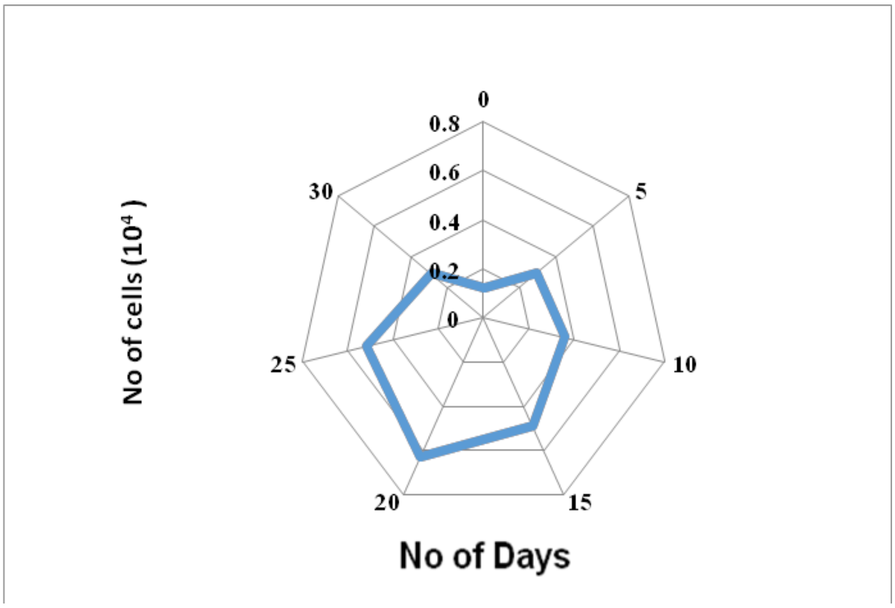

Figure: 7 Effect of dry biomass of *Skeletonemacostatum* in f/2 medium

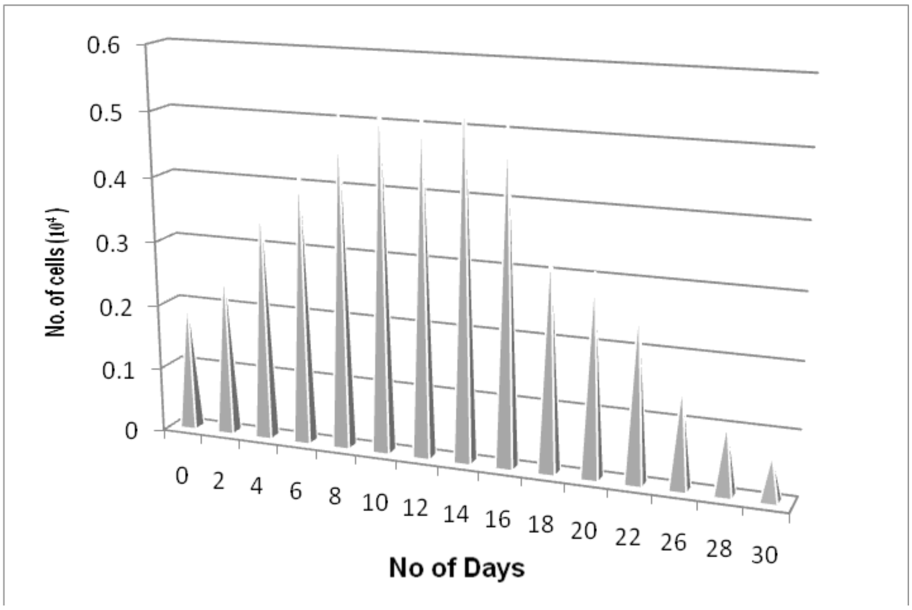

Figure:8 Growth curve of *Skeletonemacostatum* in f/2 media

4. CONCLUSION

The work presented here is only to study for bio diesel production at different stress conditions from micro algae especially the diatoms. It was seen that at different stress conditions in diatom cultures shown that the growth and lipid content at different light regimes and temperature conditions. The optimum light for the diatoms growth was observed at 16hours light and the 8 hours dark, and the optimum growth temperature was observed at 20°C on 15th day. The Specific growth rate, Division time and Generation time was calculated and mentioned in table no: 1.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

1. Griffiths MJ and Harrison STL. Lipid productivity as key characteristic for choosing algal species for bio diesel production. J Applphycol 21. 2009; 493-507.
2. Lee JY, Yoo C, Jun, SY, Ahn CY, Oh HM. Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 2010; 101: S75-S77.
3. Sournia A. Phytoplankton manual. UNESCO, Paris. 1978; 337.
4. Pratt, DM 1965. The winter-spring diatom flowering in Narragansett Bay. Limnol. Oceanogr. 10: 173 184.
5. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 1987; 148: 350–382.
6. Paffenhöfer, GA. 2002. An assessment of the effects of diatoms on planktonic copepods. Mar. Ecol. Prog. Ser., 227: 305 310.

7. Surendhiran D, Vijay M. Effect of various pretreatment for extracting intracellular lipid from Nannochloropsis oculata under nitrogen replete and deplete conditions, ISRN Chemical engineering, DOI: 10.1155/2014/536310. 2014;
8. Guillard RR. Ryther JH Studies of Marine Planktonic Diatoms. 1962; 1 Cyclotella Nana Hustedt, and Detonula.
9. Levasseur M, Thompson PA, Harrison PJ. Physiological acclimation of marine phytoplankton to different nitrogen sources. J. Phycol. 1993; 29: 587-595.
10. Venkataraman, GS 1939; a systematic account of some South Indian Diatoms. Proceedings of the National Academy of Science, India, Sect. B10 6: 293-368.
11. Hu Q, Sommerfeld M, Jarvis M, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerol as feedstocks for biofuel production: prespectives and advances. Plant J., 2008; 54: 621-639.
12. Subramanian R. A systematic account of the marine plankton diatoms of the Madras Coast. 1946; Proc. Indian Acad. Sci. 24: 85-197.
13. Ho S H, Chen C N N, Lai Y Y, Lu W B, Chang J S. Exploring the high lipid production potential of a thermos tolerant microalga using statistical optimization and semi-continuous cultivation. Bioresour. Technol. 2014; 163: 128–135.
14. Prescott G.W. How to know freshwater algae (Ed. H.E. Jaques).1954; W.H .C. Brown company publishers. Lowa. 272.
15. Converti A, Casazza A A, Ortiz E Y, Perego P, Borghi M D. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem. Eng. Proc. 2009; 48: 1146-1151.
16. Richmond A. GrobbelaarJ.U. Factors affecting the output rate of Spirulina platensis with reference to mass cultivation. 1954; Biomass. 10 4: 253-64.
17. Lv J M, Cheng L H, Xu X H, Zhang L, Chen H L. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresour. Technol. 2010; 101: 6797-6804.
18. Tesser MB Camerio DJ. Portella MC.Co-feeding of pacu (Piaractos mesopotamicus, Holmers, 1887). 2005; Larvae with Artemia nauplii and microencapsulated diet. Journal of Applied Aqua culture. 17: 47-59.
19. Khotimchenko S V, Yakovleva I M. Lipid composition of red alga Tichocarpus crinitus. Exposed to different levels of photon irradiance Phytochemistry. 2005; 66 1: 73-79.
20. Talling JF.An experimental study of the growth and photosynthesis of some fresh water plankton diatoms. 1953;Ph.D. thesis, University of Leeds.

21. Yang X. Liu P. Hao Z. Shi J. Zhang. 2012; Micro algal stains for biofuel.
22. Sohi S M H, Eghdami A. Biodiesel production using marine microalgae Dunaliella salina. J. Bio. & Env. Sci. 2014; 4(2): 177-182.
23. Rodolfi L, Chinizitelli G, Bassin, Padavani G, Biondi N, Bongi G, and Tredici M R, Micro algae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low cost photo bio reactor. Bio technol. Bioeng, 102 2009 100-112.
24. Bio resources, 7 1: 686-695. Khotimchenko, S.V. Yakovleva, IM. Lipid composition of red alga Tichocarpuscrinitus.2005; Exposed to different levels of photon irradiance. Phytochemistry, 66 1: 73-9.
25. Chen GQ. Jiang Y. Chen F. Variation of lipid class composition in Nitzschia laevisas a response to growth temperature change.Food Chem. 2008; 109:88-94.
26. Milne et al., 1990; Ginzburg; 1993; Dote Y S, Sawayama S, Inoue T, Minowa, Yokoyama S. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermo chemical liquefaction. Fuel. 1994; 73: 1855-1857.
27. Scurlock et al., 1993; Kosaric and Velikonja 1995
28. Sanjay K R, Nagendra Prasad M N, Anupama S, Yashaswi B R, Deepak B. Isolation of diatom Navicula cryptocephala and characterization of oil extracted for biodiesel production. Afr. J. Environ. Sci. Technol. 2014; 7(1): 41-48.
29. Li y, Horsman M, Wu N, Lan C Q and Calcro N D, Biofuels from Microalgae Bio technol. Prog., 24 2008 815-820.
30. Akkerman I, Janssen M, Rocha J, Wiffels R H. Photobiological hydrogen production: phytochemical efficiency and bioreactor design. Int. J. Hydrogen Energy. 2002; 27: 1195-1208.
31. Tomas, JH 1996. Effects of temperature and illuminance on cell division rates of three species of tropical oceanic phytoplankton. J. Phycol., 2: 17 22.
32. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. Primary production of the biosphere: integrating terrestrial and oceanic components. Science. 1998; 281: 237-240.
33. Lund, JWC. 1949 Studies on Asterionella: The origin and nature of the cells producing seasonal maxima. J. Ecol., 37: 389 419.
34. Hoshiai, G., Suzuki, T., Kamiyama, T., Yamasaki, M., Ichimi, K. 2003. Water temperature and salinity during the occurrence of Dinophysis fortii and D. acuminate in Kesennuma Bay, northern Japan. Fish. Sci., 69: 1303 1305.
35. Desikachary TV. Marine Diatoms from the Arabian Sea and Indian Ocean. 1987; IV.

36. Aparna G., A study of micronutrients in soils of different places around Indore, MP, India, Res. J. Chem. Sci., 5, 3, 53-56 2015
37. Al-Kandari M, Faiza Y, Al-Yamani, Al-Rifaie K. Marine Phytoplankton Atlas of Kuwait's Waters, Kuwait Institute for Scientific Research, Kuwait. 2009; 16-344.
38. Nita R., Effect of nutrient depletion and temperature stressed on growth and lipid accumulation in marine – green algae Nanochloropsis sp., Americal J Res. Communication., 2013