National Journal of Life Sciences, **Vol. 17(1) June 2020 : 33-36** ISSN : 0972-995X (Print), 2321-7960 (Online)

MEDIA OPTIMIZATION FOR CULTIVATION OF FRESHWATER MICROALGA *SCENEDESMUS* SP. UNDER CONTROLLED CONDITIONS

VINAY DWIVEDI[1], SHYAM PRASAD[1], SANTOSH KODGIRE[1], DEBANJAN SANYAL[1*] and SANTANU DAS GUPTA[2]

[1]*Research and Development, Reliance Industries Ltd., Jamnagar (Gujarat)*
[2]*Research and Development, Reliance Corporate Park, Ghansoli, Mumbai (M.S.)*

ABSTRACT : Microalgae are photoautotrophic organisms found in diverse habitat of fresh water as well as sea water and have several applications in aquaculture, nutraceuticals and CO_2 mitigation etc. Microalgae can also be utilized in production of biofuels, lipids, proteins and many high value chemicals. The large-scale production of biofuels and other value-added products from algae has several challenges and the most effective growing media with a robust contamination control strategy are critical for economic production of algal biomass. In this study, media recipes were optimized for cultivation of *Scenedesmus* sp. under laboratory conditions. Three media were used with and without 0.25% sodium bicarbonate. The results revealed that the Bold's Basel Media (BBM) with and without sodium bicarbonate were most effective for the growth of *Scenedesmus* sp. and the highest growth (4.51 OD) was observed in BBM with sodium bicarbonate followed by (4 OD) BBM media without addition of sodium bicarbonate. Both media recipes yielded statistically higher biomass growth compared to other media. The nutrient consumption and Fv/Fm data of *Scenedesmus* sp. in BBM with sodium bicarbonate was comparable with the BBM without sodium bicarbonate containing media. As path forward these media compositions are required to be validated at larger scale outdoor.

Key words : *Media optimization, Microalga cultivation. Scenedesmus sp.*

INTRODUCTION

The potential of microalgae to produce high value compounds and energy is widely recognized. These organisms are more efficient in utilization of solar energy as compared to higher plants (Chisti,2007 and Randrianarison & Aqeel, 2017). Lipids are the major components found in microalgae, significantly in green algae; however, the production ability of lipids were varying from species to species (Wahidin et al.,2013). The growth of microalgae is influenced by various environmental and physiological factors such as light intensity, photoperiod, temperature, nutrient compositions etc. (Wahidin *et al.*,2013).

Numerous media compositions have been developed worldwide for the isolation and cultivation of algae. Various permutations, or entirely new attempts, may be necessary to develop more effective growth media for algal species. However, these are based on species habitat or the specific nutrient requirements (Anderson,2005). Several strategies have been applied to improve microalgae growth and biochemical compositions. These include optimization of the media compositions (*e.g.* type of carbon source, nitrogen source, vitamins, salts, nutrients etc.), physical parameters (e.g. pH, temperature, light intensity etc.) and type of metabolism e.g. phototrophic, heterotrophic, mixotrophic, photoheterotrophic growth etc. (Mata *et al.*,2010). Among these strategies, optimization of media compositions has been reported to influence the biochemical composition of microalgae, which considerably impacts on the production of valuable compounds (Bleakley and Hayes,2017). It was earlier reported that the amount of algal biomass is altered by the addition of several nutrient dosages (Mandalam and Palsson, 1998). Therefore, it is important to optimize appropriate media composition to achieve higher yield of value-added products from microalgae. In this study, different media combinations were studied for the cultivation of *Scenedesmus* sp. such as BBM, f/2 and UPA (Urea phosphoric acid) media with and without 0.25% sodium bicarbonate.

MATERIAL AND METHODS

Media preparation : The growth of *Scenedesmus* sp. was evaluated in three different media such as BBM, f/2 and UPA with and without 0.25% sodium bicarbonate. All media components used for the study were autoclaved at 121°C and 15 lbs pressure for 20 min. The culture was inoculated in 250 ml flask containing 100 ml autoclaved media. The initial cell density was adjusted around 0.45 OD after mixing with autoclaved media. All the inoculated flasks were incubated for eight days under controlled conditions in Kuhner shaker (light intensity of 250 µE, light/dark cycle 12:12 h; 27 °C temperature; 3% CO_2, 120 rpm and 70% humidity). The samples were withdrawn for the analysis of optical density (OD) and microscopy. Total nitrogen (TN) and Fv/Fm were determined at start and end of the experiment.

BBM media composition : BBM media was prepared by $NaNO_3$:0.25 g/l; $MgSO_4.7H_2O$:0.075 g/l; NaCl:0.025 g/l; K_2HPO_4:0.075 g/l; KH_2PO_4:0.175 g/l; $CaCl_2.2H_2O$:0.025 g/l, BBM trace metals and vitamin solutions:1 ml/l.

f/2 media composition : f/2 media was prepared by $NaNO_3$: 0.75 g/l; $NaH_2PO_4 \cdot H_2O$:0.005 g/l; $Na_2SiO_3.9H_2O$:0.03 g/l, f/2 vitamin solutions:0.5 ml/l and f/2 trace metals:1 ml/l.

UPA media composition : UPA media was prepared by lab grade urea:0.45 g/l; phosphoric acid:0.31 ml/l (1N) and f/2 trace metals:1 ml/l.

*Corresponding author (email : debanjan.sanyal@ril.com) Received 18.03.2020 Accepted 25.05.2020

Growth evaluation and nutrient consumption estimation : The growth of *Scenedesmus* sp. in various media sources were determined by measuring optical density with specific growth rate and doubling time. The nutrient consumption of *Scenedesmus* sp. was also studied by analyzing the total nitrogen (TN) consumption data. The health and survival status of *Scenedesmus* sp. in various media were analyzed by measuring Fv/Fm value and microscopic observations.

Optical density (OD) analysis using spectrophotometer : The growth of *Scenedesmus* sp. was measured by OD. Spectrophotometry is a suitable indirect method to record the OD value, which is represented in terms of transmittance and can be determined by Beer-Lambert law of absorbance (Adrien,1998). Before taking OD, blank media was used for adjusting to zero. Samples OD measurement was performed at 750 nm using UV visible spectrophotometer (Shimadzu Model UV-1800).

Determination of growth rate : The growth rate of *Scenedesmus* sp. grown in different media were measured using following formula, Specific growth rate, $\mu = \ln (OD2) - \ln (OD1)/T2-T1$.

Where, OD1 & OD2 are optical densities at time T1 & T2 respectively.

Determination of doubling time : The doubling time of *Scenedesmus* sp. grown in different media were measured using following formula (Dagley & Hinshelwood,1938; Monod,1942 and Powell,1956).

$Tg = \ln (2) \times \mu^{-1}$

Where μ is specific growth rate, ln (2) is equal to 0.693, hence generation time (Tg) can be calculated in days with the following equation :

$Tg = 0.693/\mu$

Measurement of total nitrogen : The TNM-L (Total nitrogen measurement) analyzer uses a catalytic combustion method (720 °C) to convert all nitrogen elements into NO_2, which were measured using chemiluminescence detector. In brief, required algae culture was centrifuged, and supernatant was collected for TN analysis using TNM-L analyzer make Shimadzu (ASTM No. D 8083-16).

Measurement of Fv/Fm : Fv/Fm was measured using Mini PAM II (Pulse Amplitude Modulation) instrument. The sample were dark adopted for 20 minutes before Fv/Fm measurements. The dark incubated sample in falcon tube was used to determine the Fv/Fm ratio. All the samples were analyzed in a set of three readings and lastly the mean values were calculated.

Microscopic analysis : The cell morphology was observed using a Nikon ECLIPSE Ci-E microscope with DS-Ri2 camera and images were captured under 40x magnification.

RESULTS AND DISCUSSION

The optical density data showed that the BBM with 0.25% sodium bicarbonate containing media exhibited maximum growth of *Scenedesmus* sp. (4.51 OD in 8th days), compared to any other media in this experiment (Fig.1). This species also grown up to 4 OD in BBM media without addition of sodium bicarbonate. Whereas, in all other media used in this experiment, the species was grown very poorly. *Scenedesmus* sp. growth in both BBM with and without 0.25% sodium bicarbonate was statistically similar up to 8th day of growth. This demonstrates similar trend with the earlier report of Chu *et al.* (1995), showed that sodium bicarbonate did not enhance the growth of *Ankistrodesmus convolutes*. Our study indicates that the both media BBM with and without sodium bicarbonate are appropriate for the growth of *Scenedesmus* sp.

The specific growth rate data of *Scenedesmus* sp. grown in various media showed that BBM media with and without sodium bicarbonate were found to be around twice as compared to the growth in other media (Fig.2). The UPA media without sodium bicarbonate showed numerically lowest growth rate of *Scenedesmus* sp.

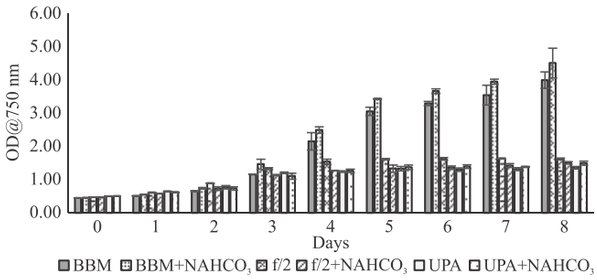

Fig. 1 Growth data of *Scenedesmus sp.* grown in various media.

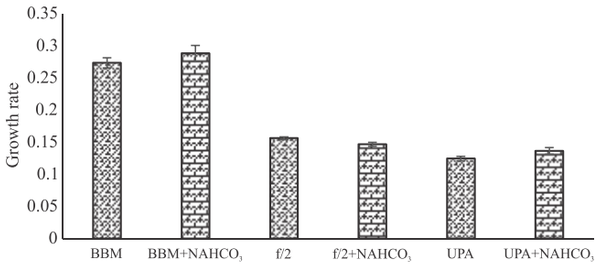

Fig. 2 Growth rate/day data of *Scenedesmus* sp. grown in various media.

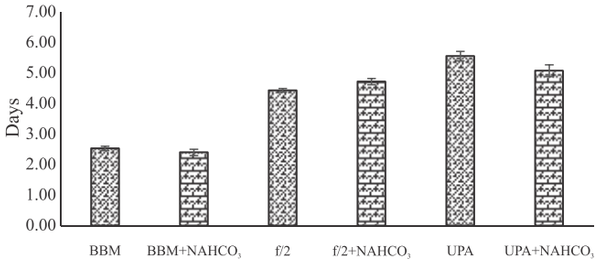

Fig. 3 Doubling time data of *Scenedesmus* sp. grown in various media.

Based on the growth rate data, the doubling time of *Scenedesmus* sp. grown in various media sources showed that BBM media with and without 0.25% sodium bicarbonate demonstrated least doubling time *i.e.* (2.41) and (2.54) respectively (Fig.3). Whereas, doubling time of species in all other media used in this experiment were found to be more. In f/2 media with and without 0.25% sodium bicarbonate showed (4.72) and (4.40) doubling time respectively. UPA media with and without 0.25% sodium bicarbonate showed doubling time of (5.08) and (5.56) respectively.

The *Scenedesmus* sp. was able to utilize nitrogen in varying amount from different cultivation media used in this experiment. TN consumption was found to be maximum (60%) in BBM media with 0.25% sodium bicarbonate followed by 51% in BBM media without sodium bicarbonate (Fig.4). TN consumption of *Scenedesmus* sp. in all other media were found to be less. The minimum TN consumption was observed in f/2 medium without sodium bicarbonate (24%). This indicates that BBM media is the most favourable nitrogen sources for the nutrient uptake and growth of *Scenedesmus* sp. (Fig.4).

The PAM data of *Scenedesmus* sp. in various media sources showed that BBM media with and without sodium bicarbonate have numerically highest Fv/Fm as compared to all other media used in this experiment. The higher values of Fv/Fm indicates the higher photosynthetic activity of cells with good health and survival status (Fig.5).

The microscopic picture of *Scenedesmus* sp. growth in BBM media confirms the presence of healthy and dividing cells (Fig.6).

The present study shows significant growth of *Scenedesmus* sp. in BBM media with and without 0.25% sodium bicarbonate demonstrated by OD increase from 0.45-4.51 and 4.0 respectively, up to 8th days under laboratory conditions. The specific growth rate in both these media were highest *i.e.* 0.288 and 0.273 respectively compared to all other media sources used. The nutrient consumption and Fv/Fm data in both these conditions were also higher than other media. The f/2 and UPA media with and without sodium bicarbonate showed significantly poor growth of *Scenedesmus* sp. under controlled conditions. As path forward, this study needs to be validated in open ponds conditions to evaluate impact of media in bigger scale outdoor.

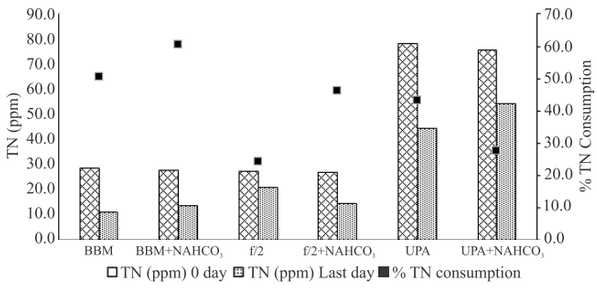

Fig. 4 TN consumption data of *Scenedesmus* sp. grown in various media.

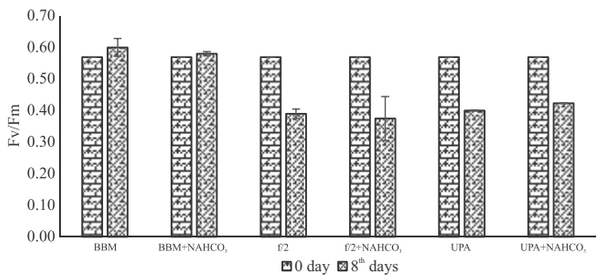

Fig. 5 PAM data of *Scenedesmus* sp. grown in various media.

Fig. 6 Microscopic observation of *Scenedesmus* sp. grown in BBM media

ACKNOWLEDGMENTS

We thank Dr. Ajit Sapre, Group President, Research & Technology, Reliance Industries Limited, for his support and Dr. Vinod Nagle for providing initial inoculum.

REFERENCES

Anderson, R. A. (2005). Algal Culturing Techniques. Academic Press Publication, West Boothbay Harbor, p. 578.

Adrien, N. G. (1998) Derivation of mean cell residence time formula. *J. Environ. Eng.*, **124(5)** : 473-474.

Bleakley, S. and Hayes, M. (2017) Algal proteins: extraction, application and challenges concerning production. *Foods*, **6(5)** : 33.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnol. Adv.*, **25** : 294-306.

Chu, W. L.; Phang, S. M. and Goh, S. H. (1995). Influence of carbon source on growth, biochemical composition and pigmentation of *Ankistrodesmus convolutes*. *J. Appl. Phycol.*, **7** : 59-64.

Dagley, S. and Hinshelwood, C. N. (1938). Physico-chemical aspects of bacterial growth. Part I. Dependence of growth of Bact. *Lactis aerogenes* on concentration of medium. *J. Chem. Soc.*, **1930** : 6

Mata, T. M.; Martins, A. A. and Caetano, N. S. (2010). Microalgae for biodiesel production and other applications : A review. *Renew Sust Energ. Rev.*, **14** : 217-232.

Mandalam, R. K. and Palsson, B. (1998). Elemental balancing of biomass and medium composition enhances growth capacity in high density *Chlorella vulgaris* cultures. *Biotechnol Bioeng.*, **59** : 605-611.

Monod, J. (1942). Research on the growth of bacterial cultures (Thesis 152 Doctorate in Natural Sciences). Hermann, Paris

Powell, E. O. (1956). Growth rate and generation time of bacteria, with special reference to continuous culture. *J. Gen. Microbial.*, **15** : 492-511.

Randrianarison, G. and Aqeel, A. M. (2017). Microalgae : A potential plant for energy production. *Geology, Ecology and Landscapes*, **1(2)** : 104-120.

Seyfabadi, J.; Ramezanpour, Z. and Khoeyi, Z. A. (2011). Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *J. Appl. Phycol.*, **23(4)** : 721-726.

Standard Test Method for Total Nitrogen and Total Kjeldahl Nitrogen (TKN) by Calculation, in Water by High Temperature Catalytic Combustion and Chemiluminescence Detection. ASTM No. D 8083-16.

Wahidin, S.; Idris, A. and Shaleh, S. R. M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. *Bioresource Technol.*, **129** : 7-11.