# The Growth Performance of *Tetradesmus obliquus* under Varied Light and Media Conditions

Jessica Joyce R. De Guia[1], Phoebe R. Diez[1], Mark Lester A. Millares[1], Daisy R. Sucaldito[1] and Dr. Dr. Emelina H. Mandia[2,3]

[1] *Graduate Student in Biology, De La Salle University 2401 Taft Avenue, Manila,0922, Philippines*
[2] *Associate Professor, Biology Department, De La Salle University 2401 Taft Avenue Manila, 0922, Philippines*
[3] *Head, Microalgal Systematics and Applied Phycology Research Unit (MSAPRU), De La Salle University 2401 Taft Avenue Manila, 0922, Philippines*

Corresponding Author: Phoebe R. Diez; phoebe_diez@dlsu.edu.ph

***Abstract:*** *Tetradesmus obliquus* is a microalga of ecological and economic importance. They are found to have potential use in biodiesel production and wastewater treatment. This study was conducted to understand the effect of differing light exposure and media conditions to the biomass production of *T. obliquus. T. obliquus* was cultured in varied growth media; TMRL with nitrate, TMRL without nitrate, and TMRL with urea. The cultures were exposed under 12/12 hr light/dark cycle and 16/8 hr light/dark cycle. Optical density was regularly checked to monitor the progress in the biomass production. The highest growth performance was observed in TMRL with nitrate in both the 12/12 hr and 16/8 hr light/dark cycles (mean value of 0.355, p= 0.049 and 0.444, p=0.00 respectively). The growth performance of cultures in TMRL without nitrate for both 12/12 and 16/8 light cycles were relatively higher than that of cultures in TMRL containing urea (mean value of 0.29543 and 0.24129; 0.23743 and 0.23357; p=0.00).

Keywords: *Tetradesmus obliquus;* Growth performance; Nitrate; Photoperiod; Urea

## 1. INTRODUCTION

Microalgae are usually found and can survive environments with harsh conditions [1]. Microalgae combine properties typical of higher plants (efficient oxygenic photosynthesis and simplicity of nutritional requirements) with biotechnological attributes properties of microbial cells (fast growth in liquid culture and ability to accumulate or secret some metabolites) represent the basis of microalgal biotechnology [18]. They are used in cosmetics, food, feed, nutraceutical and pharmaceutical purposes [2], [8]. They are also a suitable candidate in the production of biofuel because of their high productivity and $CO_2$ fixation capability [3].

*Tetradesmus obliquus* includes few species of microalgae that are cultured commercially. *T. obliquus* is a unicellular colonial green alga that thrives in freshwater environment. They are ecologically important as major producers of biomass in aquatic systems [15]. In addition, they have potential use in biodiesel production [6] and wastewater treatment [17].

Main environmental factors such as pH, temperature, amount of light, light cycle and nutrients plays a significant role in the algal growth and its chemical composition [10]. Among these factors, modification of nutrients and light has a great influence in the biomass production of microalgae. Changing the media composition has an effect in growth performance of microalgae. Nitrogen, aside from phosphorus and potassium is one of the macronutrients in microalgae that are essential for their growth capabilities. Among all the nutrients present in medium, nitrogen is known to have the strong influence in the metabolism of various microalgae.

.

With this, previous studies were conducted to find an alternative nutrient source of nitrogen. Danesi (2002) used urea as an alternative nitrogenous source and found that it can increase the growth rate of *Spirulina* [5]. However, Goswani, (2011) urea as a replacement for nitrogen significantly decreased the algal biomass of *S. dimorphus* and *S. quadricauda* [9]. This experiment was conducted in order to know the effect of urea as a nitrogenous source for *T. obliquus.*

Thus, when there is a nitrogen deficiency in the growth medium of microalgae, the biomass production will stop and eventually decline [4], [7].

Light condition is the main factor affecting microalgal physiology and the most crucial factor affecting photosynthesis [18].Varying light exposure was also tested in this research to determine if there is a significant difference in the growth performance of *T. obliquus* between the 12/12 hr and 16/8hr light/ dark cycles.

## 2. METHODOLOGY

*T. obliquus* inoculum was obtained in Microagal Systematics and Applied Phycology Research Unit (MSAPRU) and maintained in TMRL medium, $30^0$C, 3000 lux for a month prior to experimentation. It is a native green alga of Carmona River within the vicinity of DLSU- STC campus in Biñan City, Laguna.

### A. Culture Medium

Varying TMRL (Tung Kong Marine Research Lab) solution was prepared and used as a culture media for the growth of *T. obliquus.* For the control, TMRL with nitrate containing Fecl2 (3g/L), Na2SIO3 (1g/L), Na2HPO4 (10g/L) and KNO3 (100g/L) was used. For the second growth condition, KNO3 was eliminated to test the growth of microalgae in the absence of a nitrogen source. For the third growth condition 0.65% of urea was utilized as an alternative to KNO3.

### B. Cultivation Condition

*T. obliquus* was cultured in 100 mL TMRL medium with 16.6% inoculum contained in cotton stoppered flasks. They were maintained in 30°C under 3000 lux light intensity using white LED lamps as a light source. The cultures were manually shaken every 2 days prior to obtaining samples for optical density and pH measurements.

The effect of photoperiod on growth of algae was determined by exposing the samples to two different photoperiods for 14 days: 12/12 hr. light/dark cycle and 16/8 hr. light/dark cycle. The set-ups were covered with black cloth to eliminate other light sources.

The cell density of the inoculum at first day of inoculation was determined by using Neubauer's haemacytometer. The inoculum was serially diluted up to 10-9 to efficiently and accurately count the cells.Growth performance of *T. obliquus* was monitored by measuring the optical densities of microalgae cultures at regular time interval of 48 hours at 750nm absorbance using GENESYS 10 UV Spectrophotometer. The pH of the samples was also monitored using pH meter.

## C. Statistical Analysis

Data were analyzed using the software SPSS. One-way ANOVA at 5% level of significance (P=0.05) and Tukey's Test were used to analyze the data.

# 3. RESULTS AND DISCUSSION

Figure 1 shows the growth performance of T. obliquus through 14 days of cultivation in the different set-ups.

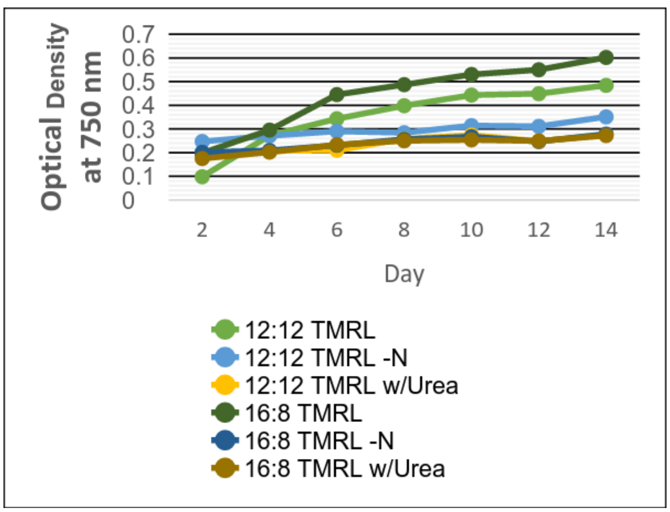


Fig. 1. The growth performance of T. obliquus under varied media and light conditions.

*T. obliquus* cultured in TMRL with nitrate exposed in 12/12hr light/dark and 16/8hr light/dark cycle yielded the highest algal biomass among the setups (mean optical density of 0.355, p= 0.049; mean optical density of 0.444, p=0.00 respectively). These results suggest that this medium condition provide the most favorable medium for *T. obliquus* since important nutrients are present in appropriate quantities.

Culture medium without nitrate resulted to lower algal biomass in both 12/12hr and 16/8hr light/dark cycle (OD= 0.295 and 0.241, p= 0.00). These results can be explained by the direct effect of nitrogen starvation on the growth of species under study. Fields et al. (2014) emphasized that the lack of required nutrients such as Nitrogen limits the capacity to synthesize proteins necessary for biomass production (e.g., cellular division) [14].

The modified TMRL medium with urea as a substitute for nitrate resulted to the lowest algal biomass. The 12/12 light/dark cycle setup obtained an optical density mean of 0.237 while the 16/8hr light /dark cycle setup obtained an optical mean density of 0.234 (both with p=0.00). In this study,
the researchers used 0.65g per liter of urea. According to Goswani (2011), the addition of amounts higher than 0.1 g/L of urea showed a significant decrease in the biomass, as well as the lipid content of *Scenedesmus dimorphus* and *Scenedesmus quadricauda* [9]. With these findings, the lowest population growth observed in TMRL with Urea is attributed to higher amount of urea utilized as compared with the previously mentioned study. The concentration of the nutrient in the medium varies according to the nutrient source used. A change in the nutrient

source might lead to an increased concentration in the medium above the tolerance level of the algal cells; such high concentrations are toxic and inhibit cell growth [13].

Table 1 ANOVA table of the growth performance of T. obliquus in exposure to 12/12hr light/dark cycle.

| 12/12hr light/dark cycle | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| TMRL w/N | 7 | .35529 | .134258 | .050745 | .23112 | .47945 |
| TMRL w/oN | 7 | .29543 | .033610 | .012703 | .26434 | .32651 |
| TMRL w/U | 7 | .23743 | .035080 | .013259 | .20498 | .26987 |
| Total | 21 | .29605 | .092448 | .020174 | .25397 | .33813 |

By comparison of the groups in 12/12hr light/dark cycle as based on the Tukey's test, it shows there is no significant difference between TMRL without nitrate and TMRL with Urea as well as TMRL with nitrate and TMRL without nitrate. However TMRL with nitrate is highly significant in comparison with TMRL with Urea.

Moreover, the comparison of the groups in 16:8hr light/dark cycle revealed by Tukey's test that the TMRL with nitrate has a significant difference compare to other medium conditions.

Table 2 ANOVA table of the growth performance of T. obliquus in exposure to 16/8hr light/dark cycle

| 16/8hr light /dark cycle | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| TMRL w/N | 7 | .44386 | .146152 | .055240 | .30869 | .57903 |
| TMRL w/oN | 7 | .24129 | .028952 | .010943 | .21451 | .26806 |
| TMRL w/U | 7 | .23357 | .033738 | .012752 | .20237 | .26477 |
| Total | 21 | .30624 | .130209 | .028414 | .24697 | .36551 |

From the results gathered, the TMRL medium resulted to highest growth performance and the TMRL with Urea has the lowest population growth.

As per result of Optical Density, Lag Phase can be observed in from 2nd -14th day in TMRL without nitrate and TMRL with Urea. On the other hand, Lag Phase in TMRL with nitrate can be observed from 2nd -6th day while Acceleration Phase was observed from 8th- 14th day wherein the population is increasing. This pattern of growth supports that TMRL with nitrate exposed under 12/12hr light/dark and 16/8hr light/dark resulted to highest algal biomass.

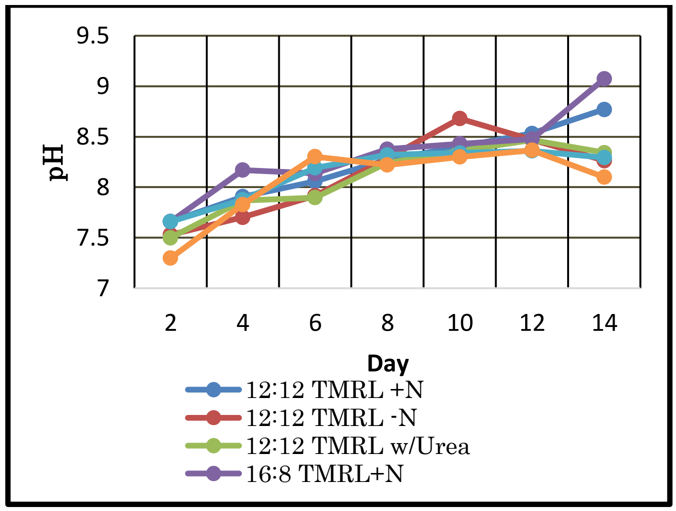


Fig. 2. The pH of T. obliquus culture through 14 days of cultivation in the different set-ups

The pH of the medium of varying set-up conditions increases as the cultivation period progresses.

## 4. CONCLUSION

The totality of the experiment revealed that *T. obliquus* cultured in TMRL with nitrate yielded the highest growth performance specifically those exposed under 16/8hr light/dark cycle (mean optical density of 0.444, p=0.00). The 16/8hr photoperiod exposure provides a better light condition for the cultivation of *T. obliquus*. Nitrogen is an important nutrient for the growth of T. obliquus as shown in the significant decrease of algal biomass in the absence of nitrogen in the culturing medium for both 12:12 and 16:8 light cycle (OD=0.295 and 0.241, p=0.00). However, urea is not an effective alternative source for nitrogen as it produced the lowest growth performance among the set-ups. *T. obliquus* cultured in TMRL with urea resulted to lowest algal biomass in both 12:12 and 16:8 light cycle (OD=0.237 and 0.234, p=0.00 respectively)

## 5. ACKNOWLEDGEMENTS

We thank Microalgal Systematics & Applied Phycology Research Unit (MSAPRU) Lab and Dr. Emelina Mandia for her shared knowledge and active guidance in doing this research.

## 6. REFERENCES

[1] S. Bauen, "A microalgae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects," Biomass Bioenergy: June 201353 pp.29-38.

[2] S. Buono, S. Langellotti, AL. Martello, A. Rinna, and V. Fogliano, "Functional ingredients from microalgae" Food Function 5:1669–1685, 2014

[3] S. Buono, A. Colucci, A. Angelini, AL. Langelllotti, M. Massa, A. Martello, V. Fogliano, and A. Dibenedetto, "Productivity and biochemical composition of *Tetradesmus obliquus* and *Phaeodactylum tricornutu*: Effects of different culcication approaches." Springer Science + Business Media Dordrecht, 2016

[4] M. Cobos, J. Paredes, D. Maddox, G. Arana, L. Flores, C. Aguilar, J. Marapara, and J. Castro, "Isolation and Characterization of Native Microalgae from the Peruvian Amazon with Potential for Biodiesel Production. Energies", 2017, 10, 224.

[5] E.D. G. Danesi, C.O. Rangel-Yagui, J.C.M. Carvalho, S. Sato, "An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis"*, 2002, Biomass Bioenerg., 23: 261–269.

[6] A. Dibenedetto, "Production of aquatic biomass and extraction of bio-oil. In: Aresta M, Dibenedetto A, Dumeignil L (eds) Biorefinery: from biomass to chemicals and fuels", 2012, Walter de Gruyter, Berlin, pp 81–100.

[7] G. Dragone, BD. Fernandez, AP. Abreu, A. Vicente, and J. Teixeira, "Nutrient limitation as a strategy for increasing starch accumulation in microalgae" , 2012, Applied energy 88 (10), 3331-3335.

[8] Z. Dubinsky, & J. Rotem, Oecologia :" *Relations between algal populations and the pH of their media", 1974,* 16: 53. doi:10.1007/BF00345087

[9] R. Goswani, "*Scenedesmus dimorphus* and *Scenedesmus quadricauda* : two potent indigenous

microalgae strains for biomass production and CO2 mitigation - A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source", 2012, Environmental Biotech Lab. Department of Biotechnology, Gauhati University. Guwahati 781014.

[10] J. M. Rousch, S. E. Bingham, M.R. Sommaerfeld,"Change in fatty acid profiles of thermo-intolerent and thermo tolerant marine diatoms during temperature stress" , 2013, J. Exp. Mar. Biol. Ecol. 295: 145-156.

[11] E. Sforza, B. Gris, C.E. De Farias Silva, T. Morosinotto, A. Bertucco, "Effects of light on cultivation of scenedesmus obliquus in batch and continuous flat plate photobioreactor", 2014, Chemical Engineering Transactions, 38, 211-216 DOI: 10.3303/CET1438036

[12] W. Wang, Y. Liu, L. Geng, Z. Yang, Y. Chen, Y , "The effect of nitrogen concentration and its interaction with light intensity on population dynamics and polysaccharide content of *Microcystis aeruginosa*", 2011, Journal of Freshwater Ecology. Vol. 26, No. 2, June 2011, 241–248.

[13] Saumya Dhup,1* Dheeban C. Kannan2 and Vibha Dhawan2, " Understanding Urea Assimilation and its Effect on Lipid Production and Fatty Acid Composition of Scenedesmus Sp. Centre for Bioresources and Biotechnology, 2016, TERI University , 10, Institutional Area, Vasant, Kunj, New Delhi 110070, India

[14] Fields, M. Hise A. Lohman E.J. Bell T. Gardner R.D. Coredor L. Moll K. Peyton B. M. Characklis G.W. Gerlach R, Sources and resources : importance of nutrients, resource allocation and ecology in microlagal cultivation for lipid accumulation, 2014, Applied Micobiol Biotechnol. 2014 ; 98 (11) : 4805-481

[15] Bellinger, E. Sigee, D.2005. Freshwater Algae (Identification and Use as Bioindicators). 1st Edition. 2013, Italian Association of hemical Engineering John Wiley & Sons, Ltd, pp 17-19

[16] Mata, T. Melo A. Meireles, S. Mendes, A. Martins A. Caetano N. Potential of microalgae Scenedesmus obliquus grown in brewery wastewater for biodiesel production ,2013, Italian Association of hemical Engineering 2013; Vol.23

[17] Abdel-Raouf , N. Al-Homaidan, A.A. Ibraheem, I.B.M. Microalgae and wastewater treatment,2012,Saudi Journal of Biological Science 2012; 19, 257-275

[18] Beanvente-Valdez, J. Ahuilar, C. Esquivel, J. Zavala, A. Montañez. Strategies to enhance the production of photosysthetic pigments and lipids in chlorophyceae species, 2015, Biotechnology Reports 10(2016) 117-125