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Growth Analysis of *Chlorella vulgaris* in varied TMRL Media and Light Exposures

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Abstract: *Chlorella vulgaris* has been widely used in many experiments due to its faster rate of growth and easy cultivation. This study compared the growth response of *C. vulgaris* under varied TMRL media (TMRL with nitrate, TMRL without nitrate and TMRL with urea) and light exposures (12/12 and 16/8 hr light/dark cycles). Growth response was monitored by optical density readings at 665nm at 2-day interval for 14 days. Growth of *C. vulgaris* was significantly higher in TMRL with nitrate compared with TMRL urea and TMRL without nitrate. Significant increase in growth was observed in the 16/8 hr light/dark cycle compared with 12/12 hr light/dark cycle. The study showed that longer photoperiods with urea in the medium, as an alternative source of nitrogen, significantly increased the growth of *C. vulgaris.*

Key Words: Growth Analysis; *Chlorella vulgaris*; Modified TMRL Medium; Light and Dark Cycle

1. INTRODUCTION

Microalgae are unicellular microorganisms that utilize light energy and carbon dioxide for photosynthesis. These organisms have higher photosynthetic efficiency compared to plants for production of biomass (Converti *et al.,* 2009*).* They are rich in protein, carbohydrates and essential fatty acids (Seyfabadi *et al.,* 2010). They can be cultured almost anywhere without compromising large portion of agricultural land that could have been used for food production (Valderramaa *et al.,* 2002).

Chlorella vulgaris has been widely used in many experiments due to its faster growth and easy cultivation. However, under general growth conditions its lipid content is only 14-30%, which would not meet the commercial requirement for diesel production. Therefore, there is a need to provide a better growth condition to achieve required biomass efficiency (Illman *et al*, 2000; Spolaore *et al.,* 2006).

Light, both duration and intensity, nutrients, temperature, pH, and salinity are factors that can contribute to the rate of growth of phytoplankton (Seyfabadi *et al.,* 2010). Therefore the search for suitable conditions in obtaining optimal growth is a

necessity.

This study aimed to compare the growth response of *Chlorella vulgaris* in modified TMRL medium under varied light and dark cycles.

2. METHODOLOGY

2.1 Inoculum

C. vulgaris inoculum was provided by the Microalgae Systematics and Applied Phycology Research Unit (MSAPRU) of De La Salle University. It was maintained in BG11 medium at 30°C, 3000 lux for 20 days prior to experimentation.

2.2 Culture Media

TMRL (Tung Kang Marine Research Laboratory) culture media was used in the study. It is composed of KNO_3 (10g / 100mL DW), Na_2HPO_4 (1g/100mL of distilled water), FECl2 (0.3g/100mL of DW) and Na_2SiO_3 (0.1g/100mL of DW) (Pachiappan *et al.,* 2015). The culture media was varied as follows: TMRL with nitrate as control, TMRL without nitrate and TMRL with Urea (0.65g/1L DW).

2.3 Cultivation Set-up

A 20mL of *C. vulgaris* was added to 100mL of the three variations of TMRL culture media. Triplicates of the culture set-ups were subjected to 12/12 hr and 16/8 hr light/dark cycles. They were maintained at 3,300 lux light intensity at 30°C for 14 days. Growth and pH were monitored at two day interval. Growth was measured by optical density readings at 665nm using the GENESYS spectrophotometer.

2.4 D. Calibration of Optical Density (OD) for Cell Density of Chlorella vulgaris

A serial dilution up to 10-5 was done in mother culture. Each dilution was subjected to Optical Density (OD) readings and haemocytometer counting (Fig. 2).

2.5 Statistical Method

The statistical method that was used in the study was Repeated Measures Analysis. The software that was used in the study was IBM SPSS Statistics version 20.

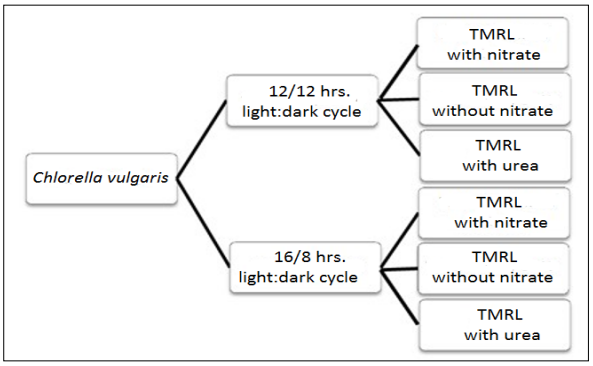

Fig. 1. Experimental Design of the Study

3. RESULTS AND DISCUSSION

Results from repeated measure analysis showed that there is a significant interaction effect between variations of light and dark cycle and medium components on optical density (p=0.68) based on table 1. It also showed that there is significant main effect of light and dark cycle (p=0.000) while medium components (p=0.126) have no significant effect on optical density.

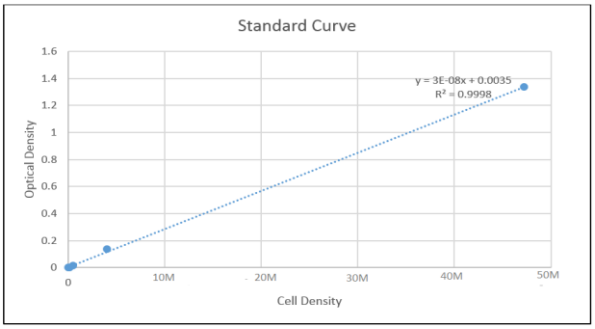

Fig. 2. Standard Curve for cell density of Chlorella vulgaris.

Growth of *C. vulgaris* was significantly higher in TMRL medium where the source of nitrogen is KNO3 compared with TMRL where source of nitrogen is urea and TMRL without nitrogen which is shown in fig. 3. Forms of nitrogen source for species of *Chlorella* was reported to have a dramatic effect in biomass and productivity.

Chlorella has an ability to metabolize urea into ammonia because of the presence of urea carboxylase enzyme before its nitrogen is incorporated into cells and bicarbonate that contributes growth and lipid production (Amin *et al.,* 2013). Deceased in nitrogen content in medium reported to increased lipid contents (Converti *et al.,* 2009; Illman *et al.,* 2000).

Table 1. Statistical analysis result of interaction effect between optical density, light and dark cycle, medium components.

Effect		Value	F	Hypothesis df	Error df	Sig.
OD	Pillai's Trace	.998	952.947[b]	5.000	8.000	.000
	Wilks' Lambda	.002	952.947[b]	5.000	8.000	.000
	Hotelling's Trace	595.592	952.947[b]	5.000	8.000	.000
	Roy's Largest Root	595.592	952.947[b]	5.000	8.000	.000
OD * Lightdarkcycle	Pillai's Trace	.930	21.345[b]	5.000	8.000	.000
	Wilks' Lambda	.070	21.345[b]	5.000	8.000	.000
	Hotelling's Trace	13.341	21.345[b]	5.000	8.000	.000
	Roy's Largest Root	13.341	21.345[b]	5.000	8.000	.000
OD * MediumComponents	Pillai's Trace	1.009	1.833	10.000	18.000	.126
	Wilks' Lambda	.178	2.197[b]	10.000	16.000	.077
	Hotelling's Trace	3.581	2.507	10.000	14.000	.057
	Roy's Largest Root	3.259	5.865[c]	5.000	9.000	.011
OD * Lightdarkcycle * MediumComponents	Pillai's Trace	1.151	2.440	10.000	18.000	.048
	Wilks' Lambda	.170	2.282[b]	10.000	16.000	.068
	Hotelling's Trace	2.999	2.099	10.000	14.000	.099
	Roy's Largest Root	2.099	3.779[c]	5.000	9.000	.040

Light exposure can also affect the growth of *C. vulgaris*. It was observed that 16 hours of light exposure had significantly increased in growth compared with 12 hours of light exposure (fig. 3). Study of Seyfabadi *et al.*, (2010) showed that *C. vulgaris* exhibits maximum growth rate at 16/8 light dark period with differentiation of pigment and protein amounts at different light cycles. Microalgae such as *C. vulgaris* manifest growth increase as higher exposure to light increase however photooxidation effect may likely to happen due to the surge of light that is not able to synthesize by the photosynthetic apparatus (Seyfabadi *et al.,* 2010).

In fig. 4, 12 hours light / 12 hours dark cycle showed highest mean OD on TMRL+N followed by TMRL-N while lowest on TMRL-Urea. However on 16 hours light / 8 hours dark cycle TMRL+N and TMRL+Urea has the highest mean values of optical density while TMRL-Urea has the lowest mean values of OD. According to the study of Choochote *et al.*, (2010) addition of Urea from a modified medium showed good growth performance of *C. vulgaris*. This was attributed to the increase of nitrogen through Urea which is responsible for rapid cell division of *C. vulgaris.*

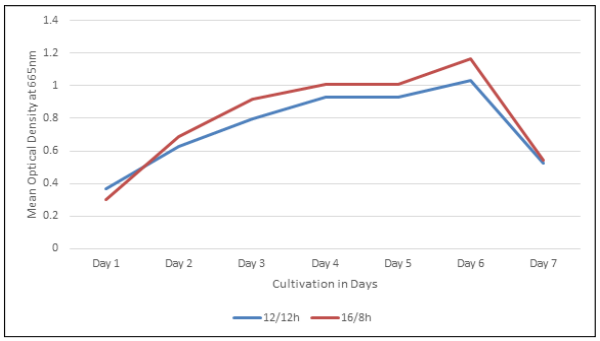

Fig. 3. Mean values of optical density at 665 nm in 12 hrs. light/12 hrs. dark and 16hrs light/8 hrs. dark cycle.

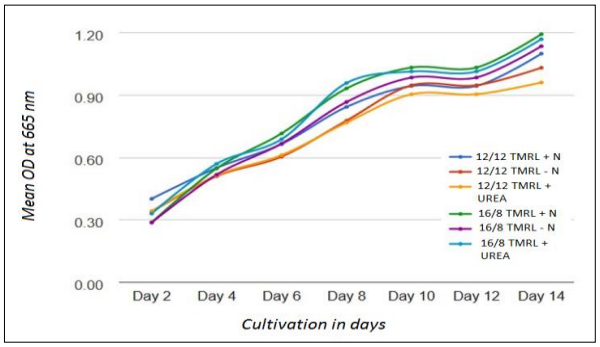

Fig. 4. Growth of *C. vulgaris* in different variation of TMRL medium and light exposure.

4. CONCLUSIONS

The longer the photoperiod (16 hours light/8 hours dark) and adding of nutrients such as urea on a minimal concentration showed good growth performance of *C. vulgaris* on a 14 day analysis. However, the removal of nitrate on the medium reduces cell density of *Chlorella* compared to the other two TMRL medium.

5. REFERENCES

Amin, N., Khalafallah, M., Abou-Sdera, S. & Matter, I. (2013).Effect of Some Nitrogen Sources on Growth and Lipid of Microalgae *Chlorella sp.* For Biodiesel Production. Journal of Applied Sciences Research, 9(8), 4845-4855.

Chisti Y, 2007. Biodiesel from microalgae. Biotechnology Advaces 0734-9750 25 294 306.

Choochote, W., Paiboonsin, K., Ruangpan, S., & Pharuang, A. (2010). Effects of Urea and Light Intensity on the Growth of *Chlorella sp*. In The 8th International Symposium on Biocontrol and Biotechnology (pp. 127-134). Ladkrabang, Thailand: King Mongkut's Institute of Technology.

Converti, A., Casazza, A., Ortiz, E., Perego, P., & Borghi, M. (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and *Chlorella vulgaris* for biodiesel production. Chemical Engineering and Processing 48, 1146–1151.

Illman, AM., Scragg, A.H., & Shales, S.W. (2000). Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme and Microbial Technology 27, 631–635

Pachiappan, P., Prasath, B., Perumal, S., Ananth, S., Shenbaga Devi, A., Kumar, S., & Jeyanthi, S. (2015). Isolation and Culture of Microalgae. Advances In Marine And Brackishwater Aquaculture, 1-15.

Seyfabadi, J., Ramezanpour, Z., & Khoeyi, Z. A. (2010). Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. Journal of Applied Phycology, 23(4), 721-726. doi:10.1007/s10811-010-9569-8.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96.

Valderramaa, L.T., Campoa, C.M.D., Rodrigueza, C.M., Luz, E., de-Bashana, b., Bashan, Y., 2002. Treatment of recalcitrant wastewaterfrom ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte Lemna minuscula. Water Res. 36, 4185–4192.