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CULTURE MEDIA OPTIMIZATION OF MARINE MICROALGA *Tetraselmis gracilis* KYLIN (BUTCHER, 1959)

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ABSTRACT

The present study was done to analyze the proximate composition of the marine microalga *Tetraselmis gracilis* in different growth phases of culture and different culture medium viz. f/2, Walne's, Chu and WC. For the study the particular species was grown in the four media and was harvested during different growth phases viz. early exponential, mid exponential, late exponential, transition and stationary. A standard protocol was developed for the culture of *Tetraselmis gracilis* under laboratory conditions which include factors such as, temperature (25°C), pH (7-8), light intensity (1500 lux) and photoperiod (12 hours light:12 hours dark). *T. gracilis* showed variation in growth kinetics and proximate composition when grown in the four media and it was found that the composition of the culture medium considerably affected the growth and biochemical composition of this microalga. Highest cell density, highest chlorophyll *a* and chlorophyll *b* content for *T. gracilis* were found in Walne's medium. In all the phase of growth highest protein and carbohydrate content were found in Walne's medium. Highest lipid content was found in Chu medium, highest moisture and ash content were found in WC medium. The results of the present study revealed that Walne's medium provides better growth and nutritional quality to *T. gracilis.* Through this it was also revealed that *T. gracilis* can be used as live feed for aquaculture organisms.

KEYWORDS: *Tetraselmis gracilis*, proximate composition, culture medium, growth phase, growth kinetics.

In the present study nutritional qualities of *T. gracilis* in different culture media were analyzed, with an intention to select a media that could provide better nutrition for the larvae in addition of supporting a good growth of algae. Studies on the use of media for modulating nutritional content are very rare. Hence the present study had great scope and of best usage in aquaculture.

MATERIALS AND METHODS

Collection of Sample and Culture Conditions

The stock of sample was collected from Central Marine Fisheries Research Institute, Cochin. The structure, size and morphology of *T. gracilis* were studied using light microscope. All the glass wares used for the culture were sterilized by autoclaving. *T. gracilis* having a cell density of 10×10^4 cells/ ml was used as inoculum. Ten percent of inoculum was inoculated in to 4 liter Haffkine's flasks containing sea water of salinity 33‰ and pH maintained at 7 (Anderson, 2005). The sea water was enriched with the four media viz. f/2, Walne's, Chu and WC. All treatments were carried out in triplicates. The cultures were continuously illuminated by fluorescent lamps at light intensity 1500 Lux, with temperature 25ºC. The biochemical analysis was carried out by harvesting the algal cells at particular growth phases viz. early exponential, mid exponential, late exponential, transition and stationary.

Standard methods were used for different analysis such as, cell count (Anderson, 2005); chlorophyll content (Strickland and Parson, 1972); protien (Lowry et al., 1951); carbohydrate (Dubois et al., 1956), lipid (Bligh and Dyer, 1959); ash and moisture (AOAC, 2005)

Statistical Analysis

The data were compared using one way ANOVA with Tukey's post hoc tests. P values of <0.05 were used as standard for statistical significance. Least significant difference (LSD) was used to compare the significant difference between means. SPSS version 22 was used for analysis.

RESULTS AND DISCUSSION

Cell Density

The study on growth of *T. gracilis* in different culture media revealed that it showed rapid cell division in the exponential phase but became reduced and constant in the stationary phase. The cell density in f/2, Walne's, Chu and WC media were 858.33×10^4 ±7.63 cells/ml, 1086.7×10^4±2.88 cells/ml, 788.33×10^4±7.63 cells/ml and 570×10^4±10 cells/ml respectively, in the exponential phase. Similar growth rate was reported by Sahay et al. (2016).

Considering the cell density, *T. gracilis* showed higher values in the Walne's medium, corroborating the results of Ender et al. (2012) and Rajeswari and Balasubramanian, (2014).

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Chlorophyll Content

In the present study highest chlorophyll *a* was found in Walne's medium (3.18µg/ml) followed by f/2 medium (2.31µg/ml), Chu medium (1.94µg/ml) and WC medium (1.72µg/ml). Same trend was also observed in the case of chlorophyll *b* content. Highest chlorophyll *b* content was found in Walne's medium (2.09µg/ml) followed by f/2 medium (1.78µg/ml), Chu medium (1.45µg/ml) and WC medium (1.12µg/ml). Similar results were reported by Lourenco et al. (1997) who cultivated *Tetraselmis gracilis* in Conway medium (3.57µg/ml).

In the present work *T. gracilis* when cultured in the four media showed more chlorophyll content in the exponential phase than in the stationary phase. This result is in accordance with the results of the studies done by Phatarpekar et al. (2000).

Proximate Composition

Protein Content

Culture of *T. gracilis* in four different media were harvested during the early exponential, mid exponential, late exponential, transition and stationary phase, analyzed for protein and the results are recorded in (T-1). Analysis using one way ANOVA revealed that there is a significant difference ($P<0.05$) in protein content in the four culture media during different phases viz., early exponential, mid exponential, late exponential, transition and stationary phase. In each medium also the protein content showed significant difference ($P<0.05$). According to the results of the present study highest protein content was found in Walne's medium in all the phases of growth viz. early exponential (33.21±0.74), mid exponential (39.90±0.60), late exponential (36.33±0.72), transition (34.29±0.54) and stationary (28.29±0.54). Lowest protein content was found in WC medium in all the phases viz. early exponential (20.26±0.74), mid exponential (26.85±0.54), late exponential (24.46±0.36), transition (23.14±0.20) and stationary (18.58±0.41).

The evaluation of protein per cell revealed maximum protein content in the exponential growth phase, decreasing throughout the growth. Fernandez-Reiriz et al. (1989) found the same trend in *Tetraselmis suecica*.

Carbohydrate Content

Culture of *T. gracilis* in four different media were harvested during the early exponential, mid exponential, late exponential, transition and stationary phase, analyzed for carbohydrate and the results are recorded in (T-1). Analysis using one way ANOVA revealed that there is significant difference ($P<0.05$) in carbohydrate content in the four culture media during early exponential, mid exponential, late exponential, transition and stationary phase. In each medium also the carbohydrate content showed significant difference ($P<0.05$). The present study revealed that highest carbohydrate content was found in Walne's medium in all the phases viz. early exponential (13.15±0.31), mid exponential (15.71±0.45), late exponential (18.09±0.15), transition (20.46±0.15) and stationary (23.78±0.40). Lowest carbohydrate content was found in WC medium in all the phases viz. early exponential (5.85±0.08), mid exponential (8.64±0.34), late exponential (10.72±0.27), transition (12.45±0.25) and stationary (15.85±0.17).

The results of the present study revealed that in all culture media carbohydrate content was found to increase from early exponential to stationary phase (Costard et al., 2012).

Lipid

Culture of *T. gracilis* in four different media were harvested during the early exponential, mid exponential, late exponential, transition and stationary phase, analyzed for lipid and the results are recorded in (T-1). Analysis using one way ANOVA revealed that there is significant difference ($P<0.05$) in lipid content in the four culture media during different growth phases. In each medium also the lipid content showed significant difference ($P<0.05$). Highest lipid content was found in Chu medium in all the phases viz., early exponential (8.57 ± 0.33), mid exponential (12.27 ± 0.30), late exponential (14.45 ± 0.33), transition (16.36 ± 0.32) and stationary (18.26 ± 0.27). f/2 medium showed lowest lipid content in early exponential phase (4.31 ± 0.17) compared with other media. WC medium showed lowest lipid content in all the phases such as, mid exponential (8.33 ± 0.34), late exponential (9.44 ± 0.24), transition (10.47± 0.12) and stationary (13.31 ± 0.13) except early exponential. In all culture media lipid content was found increased from early exponential to stationary phase. In present work *T. gracilis* showed an increase in lipid content as the culture age. This result was also supported by Huerliman et al. (2010) for *Tetraselmis* sp. harvested during logarithmic, late logarithmic and stationary phases, grown in L1, f/2 and K medium.

Moisture Content

Culture of *T. gracilis* in four different media were harvested during the early exponential, mid exponential,

late exponential, transition and stationary phase, analyzed for moisture and the results are recorded in (T-1). Analysis using one way ANOVA revealed that there is significant difference ($P<0.05$) in moisture content in the four culture media during early exponential, mid exponential, late exponential, transition and stationary phase. In each medium also the moisture content showed significant difference ($P<0.05$). Accordig to the results of the present study highest moisture content was found in WC medium in all the phases viz. early exponential (43.54±0.40), mid exponential (38.59±0.32), late exponential (35.61±0.28), transition (32.76±0.46) and stationary (28.44±0.51). Lowest moisture content was found in Walne's medium in all the phases viz. early exponential (22.48±0.23), mid exponential (16.68±0.26), late exponential (14.70±0.17), transition (12.46±0.24) and stationary (10.27±0.10). In all culture media moisture content was found to decrease from early exponential to stationary phases.

Ash Content

Culture of *T. gracilis* in four different media were harvested during the early exponential, mid exponential, late exponential, transition and stationary phase, analyzed for ash content and the results are recorded in (T-1). Analysis using one way ANOVA revealed that there is significant difference ($P<0.05$) in ash content in the four culture media during early exponential, mid exponential, late exponential, transition and stationary phase. In each medium also the ash content showed significant difference ($P<0.05$). According to the results highest ash content was found in WC medium in all the phases viz. early exponential (13.61±0.18), mid exponential (16.49±0.20), late exponential (20.27±0.09), transition (22.64±0.09) and stationary (24.78±0.16). Lowest ash content was found in Chu medium in all the phases viz. early exponential (14.41±0.28), mid exponential (11.25±0.08), late exponential (10.76±0.08), transition (8.57±0.04) and stationary (6.29±0.08). In all culture media except Chu medium ash content was found increased from early exponential to stationary phases. This result was supported by Costard et al. (2012) and Krishnan et al. (2016).

The results of the present study revealed that Walne's medium provides better growth and nutritional quality to *T. gracilis*. So Walne's medium can be considered as the best medium for providing better growth and nutritional quality to *T. gracilis*. By analyzing the results it can be confirmed that, Walne's medium provide better growth and metamorphosis for bivalve mollusks and shrimp larvae. Thus it can be used as live feed for aquaculture organisms.

Table 1: Proximate composition of *Tetraselmis gracilis* grown in four media during diferent growth phases of culture.

Medium	Growth phases	Protein	Carbohydrate	Lipid	Moisture	Ash
f/2 medium	Early exponential	30.09±0.54	9.30±0.30	4.31±0.17	35.20±0.25	8.40±0.30
	Mid exponential	35.13±0.54	12.93±0.31	9.07±0.06	30.47±0.49	10.29±0.15
	Late exponential	33.69±0.54	16.26±0.17	11.56±0.50	26.65±0.40	11.55±0.34
	Transition	28.89±0.74	18.14±0.14	13.57±0.51	24.54±0.29	13.32±0.22
	Stationary	20.74±041	22.34±0.49	15.62±0.53	20.91±0.23	16.57±0.16
Walne's medium	Early exponential	33.21±0.74	13.15±0.31	5.58±0.33	22.48±0.23	13.22±0.20
	Mid exponential	39.90±0.60	15.71±0.45	8.62±0.17	16.68±0.26	17.02±0.14
	Late exponential	36.33±0.72	18.09±0.15	9.56±0.41	14.70±0.17	18.83±0.16
	Transition	34.29±0.54	20.46±0.15	12.94±0.45	12.46±0.24	19.71±0.14
	Stationary	28.89±0.54	23.78±0.40	16.11±0.40	10.27±0.10	22.31±0.17
Chu medium	Early exponential	24.18±0.53	6.91±0.12	8.57±0.33	40.26±0.23	14.41±0.28
	Mid exponential	30.21±0.36	9.60±0.14	12.27±0.30	36.03±0.39	11.25±0.08
	Late exponential	28.29±0.54	12.27±0.17	14.45±0.33	33.27±0.30	10.76±0.08
	Transition	26.49±0.54	14.17±0.12	16.36±0.32	30.06±0.25	8.57±0.04
	Stationary	18.82±0.54	17.66±0.12	18.26±0.27	26.09±0.41	6.29±0.08
WC medium	Early exponential	20.26±0.74	5.85±0.08	6.39±0.12	43.54±0.40	13.61±0.18
	Mid exponential	26.85±0.54	8.64±0.34	8.33±0.26	38.59±0.32	16.49±0.20
	Late exponential	24.46±0.36	10.72±0.27	9.44±0.24	35.61±0.28	20.27±0.09
	Transition	23.14±0.20	12.45±0.28	10.47±0.12	32.76±0.46	22.64±0.09
	Stationary	18.58±0.41	15.85±0.17	13.31±0.13	28.44±0.51	24.78±0.16

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REFERENCES

Anderson R.A., 2005. Algal culturing techniques. Elsvier Academic Press, USA: **154**, 239- 639.

AOAC, 2005. Association of Analytical Chemists. In: Official methods of analysis of AOAC international 18th edition. Edited by W. Horwitz and G.W. Latimer. (AOAC International), Gaithersburg.

Bligh E.G. and Dyer W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys., **37**: 911-917.

Costard G.S., Machado R.R., Barbarino E., Martino R. and Lourenco S.O., 2012. Chemical composition of five marine microalgae that occur on the Brazilian coast. IJFAS, **4**(9): 191-201.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F., 1956. Colorimetric method of sugars and related substances. Anal. Chem. **28** (3): 350-356.

Endar V., Sarjito., Hutaberat J. and Prayitno B., 2012. Effect of using Guillard and Walne technical culture media on growth and fatty acid profiles of microalgae *Skeletonema* sp. in mass culture. J. Coast. Dev., **16** (1): 50-56.

Fernandez-Reiriz M.J., Perez-Camacho A., Blanco J., Planas M., Campos M.J. and Labarta U., 1989. Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. Aquaculture, **83**: 17-37.

Huerliman R., de Nys R. and Heiman K., 2010. Growth, lipid content, productivity and fatty acid composition of tropical microalgae for scale up production. Biotechnol. Bioeng., **107:** 245-257.

Krishnan A., Joseph A. and Ganga R.S., 2016. The diatom *Nitzschia palea* as a potential live feed for ornamental fish and molluscan hatchery. In: Marine Biodiversity and Bioprospecting for Sustainable Livelihood (Joseph, A., Philip, R.) pp: 286- 301. Cochin University of Science and Technology Cochin.

Lourenco S.O., Marquez U.M.L., Marcini-Filho J., Barbarino E. and Aidar E., 1997. Changes in biochemical profile of *Tetraselmis gracilis* I. Comparison of two culture media. Aquaculture, **148**: 153-168.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., **193**(1): 265-275.

Phatarpekar P.V., Sreepada R.A., Pednekar C. and Achuthankutty C.T., 2000. A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures. Aquaculture, **181**: 141-155.

Rajeswari M.V. and Balasubramanian T., 2014. Comparative study on the growth of *Skeletonema costatum*: a microalga as live feed for aquaculture importance. International Journal of Research in Fisheries and Aquaculture, **4**(3): 117-121.

Sahay M.I.S., Arunachalam K., Nair B.B. and Jayalakshmy, 2016. Biochemical characterization of eight marine microalgae grown in batch cultures. JABU., **7**(3): 19- 41.

Strickland J.D.H. and Parsons T.R., 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada, ed.2, Ottawa. 185-192.