CULTIVATION OF *TETRASELMIS STRIATA* UNDER OPTIMIZED GROWTH CONDITIONS AND BIOMASS QUALITY EVALUATION FOR FISH FEED PRODUCTION

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Abstract

The marine microalga *Tetraselmis striata* was cultivated in drilling waters obtained from the commercial fish farm Plagton S.A. (western Greece). The waters were supplemented with the fertilizer Nutri-Leef 30-10-10 of which the optimum growth quantity was found to be 0.2 g L^{-1}. The effect of different pH and temperature values on biomass and lipid yields was examined. The optimum growth conditions were found to be pH 8 and temperature 25 ^{o}C and lead to maximum biomass productivity of 79.8 mg L^{-1} d^{-1} and a specific growth rate of 0.16 d^{-1}. The biomass of *Tetraselmis* produced in the optimum growth conditions was rich in bioactive compounds and especially crude protein (51.3%), while analysis of the fatty acids revealed high percentages (up to 14%) of eicosapentaenoic acid (EPA).

Keywords: *substrate optimization, high value products*

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1. Introduction

Aquaculture is one of the most fast growing food industries worldwide and faces significant challenges including the high costs of fishmeal and fish oil for inclusion in the diets of farmed fish. Microalgae are a proven source of protein, lipids, carbohydrates and vitamins, and can improve fish growth and fillet quality. The nutritional value of microalgae is determined mainly by their protein and polyunsaturated fatty acid (PUFA) content (e.g., EPA, arachidonic acid (ARA), and docosahexaenoic acid (DHA)). Strategies such as light intensity, composition of growth media, pH and temperature manipulation, permit both lipid composition modulation and optimization of overall lipid yield.

In this study, the microalga *Tetraselmis striata* was cultivated in drilling waters obtained from the commercial fish farm Plagton S.A.. The drilling waters had natural salinity of 29 ‰ and no nutrient load, thus mineral supplements are essential for microalgal growth. Aiming to minimize production costs that could enable full-scale cultivation, the commercial fertilizer Nutri-Leef (30% -TN, 10% -P, 10% -K) together with an additional inorganic carbon source ($NaHCO_3$) was employed as growth medium for *Tetraselmis*. The quantity of fertilizer required to enrich the growth medium was recorded, as well as effects of pH and temperature on the specific growth rate and the ability of the strain to biosynthesize bioactive metabolic products.

2. Material and Methods

Samples of 50 mL were taken every 48 hours to determine growth and intracellular products. Biomass productivity, expressed in mg $L^{-1}d^{-1}$ and maximum specific growth rate (d^{-1}) estimated according to Tsolcha *et al.* (2017). Intracellular carbohydrates were determined by the Dubois method (1956), while lipids were extracted from biomass following Folch's method (1957). Crude protein content was determined with Kjeldahl method following AOAC (1995) and amino acid composition of the lyophilized biomass was carried out according to Kotzamanis *et al*. (2018). Finally, pigment concentration in the wet biomass was estimated applying the equations given by Lichtenthaler & Buschmann (2005), using acetone with 20% (v/v) water as the solvent.

3. Results and Discussion

Initially, the optimal quantity of fertilizer for the highest specific growth rate and biomass productivity of *T. striata* was determined. Quantities of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 g Nutri-Leef per litre were tested at 25±1ºC ºC, under uncontrolled pH, while 0.18 g L^{-1} of $NaHCO_3$ was added into all experimental sets. The results showed that high biomass productivities were achieved for both 0.1 and 0.2 g L^{-1} of fertilizer (106.8 and 134.6 mg $L^{-1}d^{-1}$) corresponding to specific growth rates of 0.15 and 0.24 d^{-1} respectively. However, for fertilizer quantities over 0.4 g L^{-1}, biomass yields gradually reduced, and showed productivities ranging from 60.8 to 92.3 mg $L^{-1}d^{-1}$ and specific growth rates of between 0.13 d^{-1} and 0.21 d^{-1}. A similar pattern was also seen for the lipid yield results, indicating that as the fertilizer increased in quantity, the lipid content decreased from 16.1 to 9.8% d/w. Thus the optimum fertilizer quantity was found to be 0.2 g L^{-1} which led to the highest biomass productivity and enhanced lipid content (16.3%). Using the optimum fertilizer quantity two different pH values of 7 and 8 were then tested and pH 8 was found to present significant biomass productivity of 79.8 mg L^{-1} d^{-1} with a specific growth rate of 0.16 d^{-1} and maximum oil content of 26.4%. The effect of temperature (at 19 ±1 and 25±1ºC) was then examined under the optimized pH conditions. The microalga grew well at both temperatures although the highest biomass yield recorded at 25±1ºC, while lipid content slightly enhanced at 19±1ºC (Table 1). Finally, the biomass of *T. striata* produced under the optimum growth conditions (pH=8, T=25ºC) was rich in lipids, carbohydrates, proteins and pigments (27.3, 18.3%, 51.3% and 3.6%, respectively). The optimized biomass presented adequate amino acid profile with high essential amino content (22.7%) and high essential to non-essential amino acid ratio (EAA/NEAA) of 0.96, which are considered suitable aquafeed ingredients. Among them the most abundant amino acids was Leucine (4.7%) and Lysine (3.4 %). In conclusion, fatty acid analysis revealed that the biomass contained 10-14% EPA, indicating its high value for incorporation into conventional fish feed.

Table 1. Effect of pH and temperature on *T. striata* biomass productivity, specific growth rate and lipid content.

Growth condition	Biomass productivity mg $L^{-1}d^{-1}$	Specific growth rate d^{-1}	Lipid content % dw	Lipid productivity mg $L^{-1}d^{-1}$
pH =7, T= 25± 1 ºC	60.1	0.14	25.0	19.1
pH= 8, T= 25± 1 ºC	79.8	0.16	26.4	17.1
pH= 8, T= 19± 1 ºC	60.0	0.190	27.0	17.8

4. Conclusions

According to the experimental results, under the optimized growth conditions the microalga *Tetraselmis striata* was able to produce high quality biomass rich in nutrients and EPA important for fish growth.

Acknowledgements

The project "Large-scale cultivation of microalgae and utilization of the biomass produced as alternative raw material in fish feed-AlgaFeed4Fish" (MIS 5045858, FK 80916), was co-funded by European (European Regional Development Fund) and National Resources (General Secretariat for Research and Technology) in the context of the Operational Program "Competitiveness, Entrepreneurship and Innovation (EPANEK, NSRF 2014-2020), specifically through the Action "Special Actions“ - "Aquacultures" - "Industrial Materials" - "Open Innovation in Culture".

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