**Cultivation of marine microalgae - Production of biomass and high value added products**

V. Patrinou1, A. Daskalaki2, C.N. Economou3 D. Bokas4, D.V. Vayenas3, G. Aggelis2 and A.G. Tekerlekopoulou1

1Department of Environmental Engineering, University of Patras, G. Seferi 2, Agrinio 30100, Greece

2Department of Biology, University of Patras, Patras 26500, Greece

3Department of Chemical Engineering, University of Patras, Patras 26500, Greece

4PLAGTON S.A., Thesi Konaki Skentou, Municipality of Xiromero, Aitoloakarnania, Greece

*Corresponding author email:* patrinou.v@upatras.gr

***Keywords:****Nannochloropsis* sp.*; Nannochloropsis oculata; Isochrysis galbana; Tetraselmis striata; substrate optimization*

**Introduction**

Marine microalgae are considered versatile cellular factories that produce a plethora of metabolic compounds. The high value added components they produce is a broad category containing mainly lipids, carbohydrates and proteins (Ma et al., 2020). Lipids from marine microalgae are significant as they are a major source of important poly-unsaturated fatty acids (PUFAs) such as omega-3 [EPA (C20:5), DHA (C22:6), α-Linolenic (C18:3(n-3)] and omega-6 (Dammak et al., 2016). Additionally, extracted high value products can be utilized in many different commercial applications including biofuels, health food supplements, aquafeeds, cosmetics, and pharmaceuticals. The addition of microalgal biomass into fish diets is beneficial for fish as it leads to improved growth and fillet quality, increased deposition of proteins in muscle tissue, improved resistance to disease, and higher fatty acid content (Shah et al., 2018).

In this work, four marine microalgae were studied (*Nannochloropsis* sp., *Nannochloropsis oculata*, *Isochrysis galbana* and *Tetraselmis striata*). Each is currently of high interest for aquafeeds and has the potential to produce important lipids. The aim was to select the most suitable microalga for full-scale production. The strains were cultivated in drilling seawaters, and specific growth rate and biomass productivity were the determining parameters for the selection of the optimum microalga. Growth medium optimization was then performed for the selected strain. Subsequent fatty acid analysis revealed significant EPA and PUFAs contents when the microalga was cultivated in the optimized growth medium.

**Materials and methods**

Dry cell biomass and lipid content were determined gravimetrically (mg L-1) as Total Suspended Solids (TSS) according to Standard Methods (1998). Biomass productivity expressed in mg L-1 d-1 was calculated following Gonçalves et al. (2016), while maximum specific growth rate (d-1) was calculated following Tsolcha et al. (2017). Lipid extraction was carried out following Folch (1957) and the fatty acid profile was determined according to AFNOR (1984).

**Results and discussion**

The microalgal strains were cultivated in 38 ‰ drilling waters originating from the commercial fish farm PLAGTON S.A. (Western Greece). The seawaters had no nutrient load and nutrient supplementation was essential to sustain growth. Only N and P, at a ratio of about 5:1, were added to the drilling waters, with the aim of reducing production costs in potential future full-scale cultivations. *Tetraselmis striata*, the Chrysophyceae *Isochrysis galbana* and the two *Nannochloropsis* species*,* were cultivated under conditions of continuous illumination of 56 μmol m−2 s−1, and unregulated temperature and pH. The biomass efficiencies achieved for the different strains are presented in Table 1.

***Table 1.*** *Biomass productivities and specific growth rates obtained from the four marine microalgae.*

|  |  |  |
| --- | --- | --- |
| Strain | Biomass productivity  (mg L-1 d-1) | Specific growth rate (d-1) |
| *Nannochloropsis* sp. | 23.2 | 0.073 |
| *Nannochloropsis oculata* | 27.0 | 0.077 |
| *Tetraselmis striata* | 40.2 | 0.101 |
| *Isochrysis galbana* | 95.2 | 0.366 |

The *Nannochloropsis* strains presented the lowest biomass productivities and specific growth rates. Although *Isochrysis galbana* exhibited the highest biomass productivity it was not selected as the optimum species, because of the difficulty in maintaining the culture’s purity. Microscopic observations showed that the strain was susceptible to contamination even in laboratory-scale experiments and thus it was not considered suitable for full-scale cultivations. Therefore, *Tetraselmis striata* was selected as the optimum species, as it presented satisfactory biomass yields under conditions of high salinity and low nutrient availability.

Substrate optimization was performed to further enhance growth. A medium of double N:P ratio (12:1) was studied in 38 ‰ and 29 ‰ seawaters. The highest biomass productivity (47.6 mg L-1d-1) was recorded in the 29 ‰ medium indicating that *T. striata* prefers lower salinities. The effect of medium composition on growth was also evaluated in 29 ‰ seawater by further enriching the medium with the commercial fertilizer Nutri-Leaf (30% TN, 10% P, 10% K) together with NaHCO3. Using Nutri-Leaf significantly enhanced the recorded biomass productivity (79.8 mg L-1d-1 with a corresponding growth rate of 0.266 d-1), while the produced biomass also contained high lipid contents of up to 27.8% dry weight.

Finally, fatty acid analysis of the biomass generated under the optimum cultivation conditions showed that *T.* *striata* produced high EPA (27.6%) and PUFAs (33.2%) contents.

**Conclusions**

The results of this study indicate that the marine microalgae *T.* *striata* is suitable for full-scale applications and can achieve significant biomass yields. Additionally, the strain is an important Pufa producer and its high nutritional value shows its suitability for incorporation into aquafeeds.

***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).

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