EFFECT OF GROWTH MEDIUM AND INORGANIC CARBON SOURCE ON GROWTH AND BIOMASS COMPOSITION OF *TETRASELMIS STRIATA*

Patrinou V.[1]*, Patsialou S.[1], Vayenas, D.V.[2], Aggelis, G.[3], Tekerlekopoulou A.G.[1]

[1] Department of Sustainable Agriculture, University of Patras, G. Seferi 2, 30100, Agrinio, Greece
[2] Department of Chemical Engineering, University of Patras, 26500, Rio-Patras, Greece
[3] Department of Biology, University of Patras, 26500, Rio-Patras, Greece

Abstract

Tetraselmis striata was cultivated in drilling water with a salinity of 2.8±0.1%. Drilling water was supplemented with two different growth substrates, a ready-to-use commercial fertilizer (Nutri-Leaf 30-10-10) and a modified chemical medium (F/2). Both growth substrates were studied with the addition of $NaHCO_3$ as the only carbon source, with pure CO_2 (100% v/v) (flow rate of 1 mL CO_2 min^{-1}) or with pure CO_2 (100% v/v) (1 mL CO_2 min^{-1}) and $NaHCO_3$, simultaneously. The effect of growth medium and inorganic carbon source was then examined on microalga's growth and biomass composition. The most favorable growth conditions were found to be Nutri-Leaf 30-10-10 and CO_2 flow rate of 1 mL min^{-1} + $NaHCO_3$, which lead to a maximum biomass productivity of 154 mg L^{-1} d^{-1} (corresponding specific growth rate of 0.477 d^{-1}) and a significant CO_2 biofixation rate (RCO_2) of 293.9 mg L^{-1} d^{-1}. At the same conditions the biomass of *T. striata* presented the highest quality (proteins 45.1% d.w., lipids 29.0% d.w., carbohydrates 16.1% d.w. and total chlorophylls 5.1% d.w.), while fatty acid analysis also revealed high percentages of eicosapentaenoic acid (EPA up to 18.2% of the total lipids). Therefore, Nutri-Leaf 30-10-10 and CO_2 flow rate of 1 mL min^{-1} + $NaHCO_3$ can be considered a promising strategy for the cultivation of *T. striata* with the purpose of producing alternative aquafeeds.

Keywords: *Growth substrate, inorganic carbon sources, high added value products, fatty acid composition*

*Corresponding author: Patrinou Vasiliki (patrinou.v@upatras.gr)

1. Introduction

Nowadays there is a constant demand for increasing amounts of fishmeal and fish oils, which are the main sources of proteins and lipids in the commercial fish feeds. The increased cost of fish feed has focused the interest of the scientific and industrial community on the development of alternative sources of these compounds. Microalgae are considered to be a versatile source of high added value products (proteins, lipids, carbohydrates, pigments, poly-unsaturated fatty acids (PUFAs) such as EPA (C20:5)). Thus, their biomass could partially replace the proteins and lipids needed for fish feed production. In this study, the microalgae *Tetraselmis striata* was cultivated employing two different growth substrates. The F/2 medium was tested as it is a medium specifically designed for the cultivation of marine microalgae, while the commercial fertilizer Nutri-Leef (30% -TN, 10% -P, 10% -K) was also studied with the aim of reducing production costs in a potential full-scale unit. The aim of the study was to evaluate both the effect of growth medium and inorganic carbon sources ($NaHCO_3$, pure CO_2) on the growth and the ability of the strain to biosynthesize high added value products. The suitability of the produced biomass for incorporation into fish feeds was evaluated based on the basis of quality analyses.

2. Material and Methods

All experiments were conducted in 4L glass aquariums under constant operating conditions of continuous illumination (56 μmol photons m^{-2} s^{-1}), pH 8±0.2 and temperature 25±1°C. Samples of 100 mL were taken to determine biomass production and intracellular products. Biomass productivity (mg L^{-1} d^{-1}) and specific growth rate (d^{-1}) were estimated according to Patrinou et al. (2022). RCO_2 was determined according to the equation of Kassim & Meng (2017). Protein content was determined using Lowry method. Carbohydrates were determined according to Dubois method (1956), while lipids were determined using the Folch's method (1957). The commercial fertilizer used was Nutri-leaf (30% TN- 10% P- 10% K) (Miller, USA), while a detailed description of its composition is provided at Patrinou et al. (2022). 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 extraction solvent.

3. Results and Discussion

In order to identify the most efficient growth substrate for *T. striata* the fertilizer Nutri-Leaf 30-10-10 (experimental set A) and a modified F/2 medium (set B) were initially studied. Based on previous experimental data an optimized fertilizer quantity of 0.2 g L^{-1} was used, while the F/2 medium was modified to have a similar N:P ratio (≈ 8:1) as Nutri-Leaf 30-10-10. Considering that carbon addition in microalgal cultures can significantly enhance biomass productivity and its metabolic components (by promoting intracellular enzymatic reactions) both substrates (A, B) were supplemented with $NaHCO_3$ (0.18 g L^{-1}) as a low- cost inorganic carbon source. According to the literature, $NaHCO_3$ can be efficiently utilized by microalgae and converted intracellularly to CO_2. The results revealed that set A with Nutri-Leaf resulted in higher biomass productivity (93.7 mg L^{-1} d^{-1}) and RCO_2 (178.3 mg L^{-1} d^{-1}), while also increasing carbohydrate, lipid and total chlorophyll accumulation (20.4% d.w., 30.2% d.w. and 5.1% d.w. respectively) compared to set B (Table 1). Experiments were then carried out with Nutri-Leaf 30-10-10 by substituting $NaHCO_3$ with pure CO_2 at a flow rate of 1 mL min^{-1} (set C). Initial experiments showed that higher flow rates of 10 or 20 mL CO_2 min^{-1} resulted in a gradual acidification of substrate's pH (7±0.5) due to the high concentration of soluble CO_2 (data not shown). Therefore, the research focused on the flow rate of 1 mL CO_2 min^{-1}. CO_2 addition in substrate C further increased biomass productivity and RCO_2 up to 120 mg L^{-1} d^{-1} and 225.1 mg L^{-1} d^{-1}, respectively. Compared to substrate A, biomass composition presented no significant differences among lipid and chlorophyll accumulation rates, however CO_2 addition favored protein production (44.1% d.w.). It should be noted that the pH was easily adjusted to 8. To avoid frequent pH adjustment, 1 mLCO_2 min^{-1} was added to the fertilizer's substrate together with $NaHCO_3$ (0.18 g L^{-1}) (set D). The combination of CO_2–$NaHCO_3$ created a buffer system that kept the pH stable. Therefore, set D recorded the highest RCO_2 (293.9 mg L^{-1} d^{-1}), which also resulted in the highest biomass production (154 mg L^{-1} d^{-1}) and intracellular compound accumulation among all growth conditions tested (Table 1). F/2 was also tested with 1 mLCO_2 min^{-1} + $NaHCO_3$ (set E). Even though biomass of set E, and its components improved compared to set B, set D achieved overall the highest yields indicating that the fertilizer is a more suitable growth substrate for *T. striata*. Finally, under the most favorable growth conditions (set D), biomass not only presented the highest quality (proteins 45.1% d.w., lipids 29.0% d.w., carbohydrates 16.1% d.w. and total chlorophylls 5.1% d.w.), but also high contents of PUFAs (35.9% of total lipid) and especially EPA (18.2% of total lipid). Therefore, the biomass produced can be considered as a suitable aquafeed ingredient.

Table 1. Effect of different growth substrate and inorganic carbon sources on biomass production, CO_2 biofixation rate and biomass composition of *Tetraselmis striata*.

Growth conditions	Biomass productivity (mg L^{-1} d^{-1})	CO_2 Biofixation rate (mg L^{-1} d^{-1})	Intracellular products (% d.w)				
			Proteins	Carbohydrates	Lipids	Total chlorophylls	Total carotenoids
A	93.7 ± 11.5	178.3 ± 15.2	38.7 ± 1.2	20.4 ± 2.2	30.2 ± 1.5	5.1 ± 1.2	0.5 ± 0.1
B	56.2 ± 10.3	99.3 ± 4.8	43.7 ± 2.8	13.6 ± 2.4	27.9 ± 3.0	3.9 ± 0.9	0.6 ± 0.1
C	120 ± 19.6	225.1 ± 16.8	44.1 ± 1.7	13.1 ± 1.8	30.1 ± 2.8	4.8 ± 0.5	0.7 ± 0.1
D	154 ± 18.3	293.9 ± 18.9	45.1 ± 0.1	16.1 ± 1.6	29.0 ± 3.2	5.1 ± 0.5	0.8 ± 0.1
E	102.7 ± 21.0	187.6 ± 22.1	42.7 ± 2.1	16.0 ± 2.1	29.0 ± 1.8	4.2 ± 0.3	0.5 ± 0.2

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