ANALYSING CULTIVATION STRATEGIES OF *HAEMATOCOCCUS PLUVIALIS* TO ESTABLISH CULTURES OF HIGH PERFORMANCE AND CARBON UTILIZATION EFFICIENCY

C. Samara[1], G. Papapanagiotou[1], C. Chatzidoukas[1,*]

[1]Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece

*(*chatzido@auth.gr)*

ABSTRACT

Inorganic carbon constitutes a critical nutrient for the growth of microalgae and in most cases of autotrophic cultivation it is predominantly provided as gaseous CO_2. However, due to its limited solubility in aqueous environments, an excess amount must be supplied to satisfy microalgae's needs, leading to low carbon utilization efficiency[1]. Sodium bicarbonate ($NaHCO_3$) emerges as an alternative source of inorganic carbon, with its dissociation in water yielding the ion HCO_3^-, which can be better utilized by microalgae[2]. This study endeavors to establish an efficient sodium bicarbonate feeding policy to photosynthetic microalgae cultures, with the dual objectives of meeting daily culture requirements and optimizing CO_2 fixation efficiency. To this end, 8-day batch experiments of *H. pluvialis* cultures in a 3-L bench top photobioreactor were designed employing two distinct carbon sources: gaseous CO_2 or a $NaHCO_3$ solution, with their respective performances compared. All experiments were conducted under the same culture conditions including consistent culture volume (2.5 L), stirring speed (250 rpm), temperature (25 ^oC), aeration rate (0.5 L min^{-1}), intensity of the peripheral lighting (18000 lux), photoperiod (16/8h light/dark) and initial culture density (150 mg/L). The used culture medium was Optimal Haematococcus Medium (OHM) modified to ensure adequate nitrogen and phosphorus concentrations throughout the cultivation period.

When gaseous CO_2 was supplied, two levels of CO_2-enriched atmospheric air were employed, either 0.5%, or 1% v/v. Conversely, for $NaHCO_3$ supplementation, two fed-batch feeding strategies were implemented, while maintaining a constant working volume of 2.5 L with simultaneous removal of culture volume equal to that of the $NaHCO_3$ solution added. Dry weight and optical density measurements were conducted daily, while macronutrients and pigments were determined every 2 days. The findings reveal that enhanced biomass production is succeeded when $NaHCO_3$ is employed as the carbon source compared to CO_2, signifying an augmented carbon bioavailability for the microalgae population. Furthermore, adopting a feeding strategy involving frequent and small $NaHCO_3$ doses, as opposed to rare and large doses, the CO_2 losses due to aeration are minimized.

KEYWORDS: *Haematococcus pluvialis*, photo-bioreactor, autotrophic cultivation, sodium bicarbonate, enhanced carbon fixation

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