

UPSCALING THE AUTOTROPHIC CULTIVATION OF THE MICROALGA *CHLORELLA SOROKINIANA* IN PILOT-SCALE PONDS: BATCH VS. SEMI-CONTINUOUS OPERATIONS

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ABSTRACT

Commercial-scale microalgae production predominantly occurs in outdoor open cultivation systems. This study deals with the large-scale autotrophic cultivation of the microalga *C. sorokiniana* in open ponds of 1000L capacity each, situated within a green house. The study demonstrates the successive scaling-up challenges encountered to attain the necessary inoculum volume while addressing issues related to reduced culture productivity and the risk of contamination. Three sequential cycles of precultures of successively increasing volume by an order of magnitude and transitioning from artificial to natural light were established involving: (i) the level of 2-L Erlenmeyer flask within a shaking incubator, (ii) the 100-L semi-pilot scale circular open pond within a laboratory setting, and (iii) the 1000-L pilot-scale open pond located within the greenhouse facilities at the University of Thessaly. These series of precultures, with the final one lasting for only five days, produced 1000L of *C. sorokiniana* culture with a density of approximately 350 mg L⁻¹ dry cell mass, sufficient for inoculating three pilot-scale ponds isometrically.

During the 14-day cultivation period, one pond operated entirely in batch mode, while the other two transitioned to semi-continuous operation on the 5th and 7th days, respectively, with 10-15% of the culture volume removed daily and immediately replenished with an equal volume of BBM^[1] growth medium. Daily monitoring of all ponds included microscopic observations and measurements of dry cell weight (DCW) and nitrogen concentration. pH adjustments to a value of 7 using a 5 M HCl solution were conducted twice daily, while sodium bicarbonate solution was added regularly as an inorganic carbon source at a dosage of 750 mg L⁻¹. For contamination control, a pH-based treatment method was employed upon detecting contaminants, primarily protozoa, following the procedure outlined by Georgiou et al^[2]. The semi-continuous operation initiated on the 5th and 7th days of cultivation resulted in final biomass productions of 687 g and 641 g (DCW), respectively, outperforming batch mode operation by 58% and 48%. The produced microalgal biomass from the three ponds exhibited nearly identical compositions, comprising 57% proteins, 22% lipids, and 16% carbohydrates, and once collected was utilized for food and feed applications.

KEYWORDS: *C. sorokiniana*, open microalgae cultivation systems, pH-based contamination control, pilot-scale microalgae cultivation, microalgae biomass for food and feed applications.

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