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OPTIMIZATION OF *Phaffia Rhodozyma* SINGLE-CELL PROTEIN PRODUCTION BY RESPONSE SURFACE METHODOLOGY

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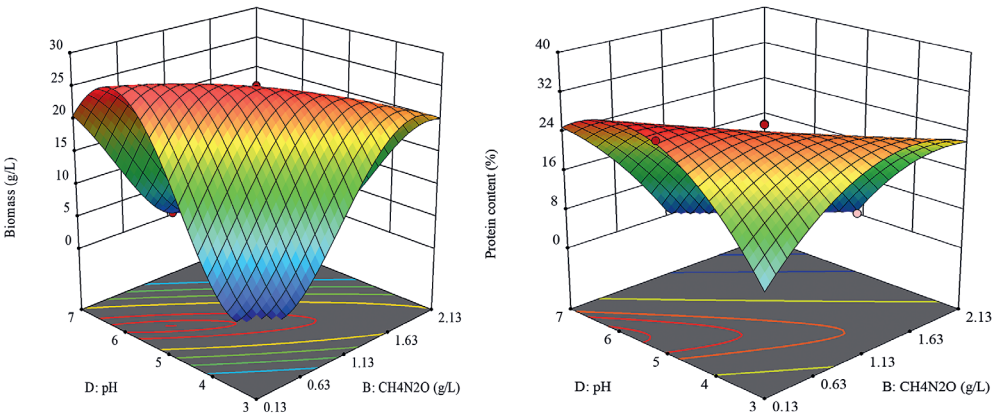
Abstract – *Phaffia rhodozyma* is widely used as a natural source of astaxanthin for aquaculture, but its protein-rich biomass remains underexplored as single-cell protein (SCP). This study aimed to reposition *P. rhodozyma* as a dual-output platform by optimizing a low-cost medium for a protein-enhanced mutant (AEC3/9) while tracking astaxanthin formation. A central composite design/response surface methodology evaluated glycerol, urea, ammonium sulfate and initial pH. Triplicate shake-flask cultivations (250 mL baffled flasks, 20 mL working volume, 22 °C, 250 rpm, 7 d) showed that protein productivity peaked on day 4, which was used as the reference timepoint for optimization; astaxanthin was modeled but not set as an optimization target. Numerical desirability indicated moderate glycerol (70–90 g/L), low urea (<0.2 g/L) and slightly acidic pH (~5.9) as favorable. The selected optimum (70.54 g/L glycerol, 0.134 g/L urea, 4.98 g/L $(NH_4)_2SO_4$, pH 5.86) yielded 26.11 g/L biomass with 29.79 % protein (7.78 g/L). Scale-up in a 5 L stirred-tank bioreactor con-firmed feasibility, reaching 33.50 g/L biomass and 6.82 g/L protein at 88 h, and indicating that protein accumulation precedes the astaxanthin-associated phase transition. Optimizing nitrogen form/level and pH enables robust production of *P. rhodozyma* biomass on glycerol, supporting its use as a scalable SCP co-product alongside astaxanthin.

Keywords – Astaxanthin co-production; effective substrate conversion; fermentation optimization; scale-up; single-cell protein



The three-dimension response surface plots demonstrated the influence of the interaction between the two independent variables on biomass (a) and protein content (b)

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