Multiscale Integration of Mixotrophic Microalgal Cultivation and Harvesting using Tap Water and Wastewater in Pilot-scale Bubble-Column Photobioreactors

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Abstract

The production of third generation biofuels and high-value co-products requires the cultivation, harvesting, and downstream processing of algal biomass. While self-shading and non-uniform nutrient distribution can limit algal growth in photobioreactors, the microalgal cell size and surface charge, and low cell density make biomass harvesting slow and expensive. Here, we employ a tightly-coupled experimental and multiscale modelling strategy for overcoming these challenges through multiscale integration of mixotrophic cultivation of *Chlorella sorokiniana*, biomass harvesting, and multiple recycling of nutrient-rich harvested broth, using tap water and municipal wastewater, in 25 L pilot-scale bubble-column photobioreactors.

This integrated process of cultivation using tap water, harvesting, and broth recycling produce biomass yields of 0.47-4.16 g/L with lipid contents of 22-29.6% w/w, and reduce the harvesting time to 13-29 minutes at a cost of 4.48-30.77 INR ($0.06-$0.42) per kg dry algal biomass, making it scalable for commercial production. The optimal inlet CO_2% using Tris, ammonium chloride, and urea in tap water are obtained as 2%, 3%, and 4%, respectively, which produce biomass yields of 4.16, 3.35, and 3.60 g/L, respectively, with lipid contents of 25.8%, 28.7%, and 27.8%, respectively, and protein contents of 50%, 51.3%, 46.7%, respectively. Using wastewater with and without media at 3% and atmospheric CO_2 produces biomass yields of 2.74, and 0.47 g/L, respectively, with lipid contents of 28.3% and 22.8%, respectively, and protein contents of 35.3% and 26%, respectively.

A multiscale model, which quantifies the transport and reaction processes at three characteristic length scales, namely the reactor (macro), the cell (meso), the organelles (micro), is employed to simulate mixotrophic algal cultivation. Our model simulations – validated using our experiments using both tap water and wastewater – quantify the autotrophic and heterotrophic components of mixotrophic growth, and predict the optimal CO_2% for maximizing biomass yield. Super-optimal CO_2 levels acidify the chloroplast, inhibit RuBisCo activity, and reduce CO_2 sequestration, while suboptimal levels produce substrate-limited growth. Acetic acid (an organic carbon source) forms acetyl-CoA in the cytosol, which enhances algal biomass and lipid synthesis. The excess acetate ions react with potash alum in the reactor to form complexes that help the microalgal cells flocculate in <30 minutes. This acetic acid-based integrated multiscale process provides a scalable technology for commercial production.

Keywords: Microalgae; *Chlorella sorokiniana*; Photobioreactor; Mixotrophic algal cultivation; CO_2 sequestration; Nitrogen sources; Macromolecules; Metabolic reactions and transport; Rapid harvesting; Potash alum; Chemical flocculation; Low-cost process; Nutrient recycling; Multiscale integration; Multiscale modelling; Wastewater treatment