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EFFECT OF DISSOLVED OXYGEN ON PHOTOAUTOTROPHIC POLYHYDROXYBUTYRATE PRODUCTION BY *SYNECHOCYSTIS SP*

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Introduction. Cyanobacteria can accumulate biopolymers as carbon and energy reserves, first as glycogen and then as polyhydroxybutyrate (PHB), under nutrient-unbalanced conditions (1,2). A major challenge in sustainable PHB production in photoautotrophs is their overall low amount of intracellular accumulation. Since glucose released during glycogenolysis also supports photorespiration (3), suppressing dissolved oxygen from photobioreactors could improve PHB concentration by reducing competition with this futile pathway. This study aimed to determine whether oxygen saturation influences PHB accumulation in a predominantly *Synechocystis* sp. consortium.

Materials & Methods. Experiments were carried out following a two-step cultivation approach in closed photobioreactors. First, *Synechocystis* sp. was grown in nutrient-abundant BG-11 until exponential growth under open conditions. Then, the resulting biomass was harvested, washed, and inoculated in nitrogen-deprived, O_2-free BG-11 to stimulate PHB accumulation. During this phase, dissolved oxygen was monitored daily and maintained at ≤ 2.0 mg O_2 L^{-1} by injecting pure gaseous N_2. A second set of photobioreactors was continuously injected with filtered air for comparison. Biomass growth was determined every 48 h in terms of optical density at 680 nm and chlorophyll at 455 nm. After harvesting and oven drying, glycogen and PHB content were monitored at selected time intervals (3, 5, and 8 d) via phenol-sulfuric (4) and acidic methanolysis/GC methods (5), respectively.

Results. Findings in our study showed that biomass growth experienced a four-fold increase under air-saturated (7.0-7.5 mg O_2 L^{-1}) and hypoxia (0.5-1.0 mgO_2 L^{-1}) conditions after 3 and 5 d, respectively (Fig. 1). Specific growth rates of both conditions were 0.194 (air-saturated) and 0.275 (hypoxia) d^{-1}. Regarding polymer accumulation, results showed that hypoxia over *Synechocystis* sp. resulted in an accumulation of 38 ± 3 mg glycogen g dried biomass^{-1} and 110 ± 10 mg PHB g dried biomass^{-1} after 8 d. However, when air-saturated conditions were tested, the cyanobacteria decreased their accumulation of both polymers achieving a maximum content of 58 ± 14 mg glycogen g dried biomass^{-1} polysaccharides and 87 ± 8 mg PHB g dried biomass $^{-1}$ on day 5. From a practical point of view, using heterotrophic bacteria (that consume oxygen) and wastewater can be a promising scenario.

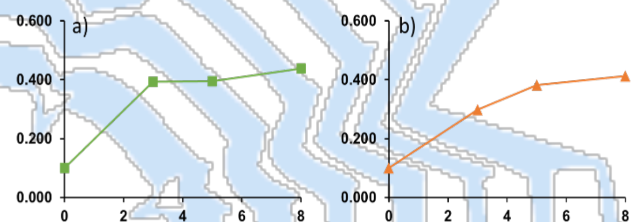

Fig. 1. Growth curve for predominantly *Synechocystis* sp. mixed consortium grown under (a) air-saturated and (b) hypoxia conditions

Conclusions. Our study demonstrated that purging dissolved oxygen from photobioreactors could enhance PHB accumulation in a predominantly *Synechocystis* sp. mixed consortium. Hypoxia conditions resulted in a significant increase in PHB production and biomass growth, while air-saturated conditions showed a decrease in both parameters. These findings provide important insights for optimizing sustainable PHB production in photoautotrophic microorganisms.

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