**Nitrate-Free Cultivation of *Aulosira fertilissima* for Low-Cost Sustainable PHB Production**

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**Abstract**

*Aulosira fertilissima* CCC444 is a diazotrophic filamentous cyanobacterium that is known to accumulate high levels of polyhydroxybutyrate (PHB), a biopolymer that is biodegradable and renewable. The production of PHB from *A. fertilissima* has attracted significant interest due to its potential applications in various industries, including bioplastics, packaging, and biomedical materials. PHB biosynthesis in *A. fertilissima* is catalyzed by the enzymes β-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase and convert acetyl-CoA to (R)-3-hydroxybutyryl-CoA, which is then polymerized into PHB. The accumulation of PHB in cyanobacteria can be influenced by multiple factors, including culture conditions, nutrient availability, and carbon source. In addition, *A. fertilissima* has also been investigated for its ability to produce PHB under stress conditions, and accumulation of PHB was observed to increase under nitrogen limitation. Besides this, the requirement for nitrogen sources for large-scale production significantly increases the cost of the process. Therefore, cultivation of *A. fertilissima* without adding nitrogen sources can lead to cost savings, environmental benefits, increased PHB accumulation, and reduced contamination risk. In this study, *A. fertilissima* was cultivated in BG11o media (BG11 without nitrate) and the cultivation conditions were optimised. Under optimized conditions, the present study showed a maximum PHB accumulation of 33.44% (dcw) after 21 days of growth followed by 0.3% acetate addition and dark incubation for 3 days. The photoautotrophic cultivation carried out in BG11o media in an illuminated bottle bioreactor resulted in biomass yield of 0.43 g L-1 leading to a maximum PHB yield of 144 mg L-1. Hence, this study demonstrates that *A. fertilissima* cells can be cultivated without nitrate which accumulated PHB under optimized conditions and this represents an important step towards the development of a sustainable and environmentally friendly approach to PHB production.

Keywords: *Aulosira fertilissima* CCC444; Polyhydroxybutyrate (PHB), Bioplastic, Cyanobacteria, Nitrate-free cultivation