A B S T R A C T

In response to problems associated with plastic wastes and their effects on the environment, there has been considerable interest in the development of biodegradable plastic materials. Polyhydroxyalkanoates (PHAs) are a group of naturally occurring biodegradable polyesters, which are found as insoluble cytoplasmic inclusions in many bacterial species. In this study, accumulation of PHAs in an unexplored diazotrophic cyanobacterium, *Aulosira fertilissima* CCC 444 was investigated. UV-spectroscopy, GC-MS, FT-IR and ^{1}H-NMR analyses confirmed that under photoautotrophic growth condition *A. fertilissima* CCC 444 accumulated the homopolymer of poly-*β*-hydroxybutyrate (PHB).

Various factors affecting PHB accumulation in *A. fertilissima* CCC 444 was investigated. The variation in one or two variable(s) at a time resulted into an enhanced PHB accumulation up to 77% dry cell weight (dcw) under P-deficiency with 0.5% acetate supplementation. Further, multivariable optimization by response surface methodology demonstrated an accumulation up to 85% (dcw) at 0.26% citrate + 0.28% acetate with 5.58 mg l^{-1} K_2HPO_4 supplementation for a culture period of 5 days. *A. fertilissima* cultures pre-grown in fructose (1.0%)-supplemented nitrate-free BG 11 medium, when subjected to the above optimized condition, the PHB pool boosted up to 1.6 g l^{-1}, a value ~50-fold higher against control.

To explore low-cost PHB production, batch-scale studies were conducted under recircultory aquaculture system in fiber reinforced plastic tanks enhanced by several manageable parameters such as sedimentation, inoculum size, mixing and culture depth. Maximum PHB accumulation in *A. fertilissima* CCC 444 was found in sedimented fish pond discharge at 20 cm culture depth with stirring and an initial inoculum size of 80 mg dcw liter^{-1}. Under optimized condition, PHB yield was reached up to 460, 445 and 399 mg l^{-1} (69, 67 and 60 g), respectively for summer, rainy and winter seasons. This study also demonstrated that *A. fertilissima* CCC 444 has high nutrient removal capacity in all seasons, maximum in summer. Nutrients such as ammonium, nitrite and phosphate reached to as low as zero level within 15 days of incubation indicating the system's high bioremediation capability.

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV) co-polymer accumulation in *A. fertilissima* CCC 444 was also investigated in presence of propionate/ valerate in the culture medium. The most significant rise in P(3HB-*co*-3HV) co-polymer content, i.e. 77% (dcw) was recorded under 0.5% fructose + 0.4% valerate supplementation. Surface analysis revealed a regular and smooth surface for P(3HB-*co*-3HV) co-polymer, against rugged and porous surface of PHB. X-ray diffraction showed semi-crystalline nature of the P(3HB-*co*-3HV) co-polymer. The thermal and mechanical properties of PHB and P(3HB-*co*-3HV) co-polymer are comparable with the bacterial polymers and the polymers obtained from other cyanobacterial sources, thus advocate their potential application in various fields.

Keywords: *Aulosira fertilissima* CCC 444, polyhydroxyalkanoates (PHAs), poly-*β*-hydroxybutyrate (PHB), recircultory aquaculture system (RAS), response surface methodology (RSM)