Optimisation of fermentation parameters for enhanced poly(3-hydroxybutyrate) production

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Over the past years, petrochemically-derived plastic wastes have been in the global spotlight due to their contribution to the rising environmental pollution. It is estimated that 367 million t plastics were globally produced, half of which are single-use items. More than 10 million t plastics are dumped into oceans from landfills every year (Kumar *et al.*, 2021). One of the great challenges is to replace conventional plastics with bioplastics with desired physical and chemical properties. Polyhydroxyalkanoates (PHAs) are natural, bio-based and biodegradable polymers that are synthesized intracellularly by various microorganisms as carbon and energy reserves (Chen *et al.*, 2011). The most well-known member of the PHAs family is poly-3-hydroxybutyrate (PHB) with similar properties to polypropylene (McAdam *et al.*, 2020). One of many reasons that impede the industrial production of PHB is the high production cost resulting in the exploration of alternative approaches in order to reduce the production cost. For this reason, crude renewable resources have been evaluated for PHB production (Pagliano *et al.*, 2017).

In this context, fruit wastes were evaluated as fermentation feedstock for PHB production in bioreactor fermentations. Fruit wastes were collected from a local market in Athens, and free sugars were extracted at 40ºC for 2 h for their utilization as fermentation feedstock. PHB production has been carried out by the bacterial strain *Paraburkholderia sacchari* DSM 17165. PHB production was carried out in a 2 L stirred-tank bioreactor (Eppendorf, Bioflo120) with 1 L working volume at 30ºC. The pH was controlled at 6.8 with 24% NH_4OH and 2M HCl solution. Aeration was 2.5 vvm, while the agitation was controlled at 1200 rpm. The effect of different carbon to inorganic phosphorous (C/IP: 32, 26.5, 24.6, 22.85) ratios was initially assessed in order to enhance PHB accumulation. Subsequently, four fed-batch fermentations were carried out at various k_La values.

The investigation of the effect C/IP ratio resulted in the highest PHB accumulation (70%, w/w) at C/IP 26.5 with a total dry weight of 90 g/L. Decreasing the C/P ratio to 22.85 resulted in lower PHB accumulation (50%, w/w). At the optimal C/P ratio (26.5), phosphorous supplementation in the feeding solution was implemented, resulting in increased total dry weight (140 g/L) with PHB accumulation of 71% (w/w). Among the k_La values used, the highest yield (0.33 g/g) and productivity (3.28 g/L/h) were observed at 206.1 h^{-1}, where the total cell dry weight was 162.6 g/L with a PHB concentration of 108.3 g/L. Decreasing the k_La 157.6 h^{-1} resulted in lower total dry weight (111.8 g/L), PHB concentration (59.1 g/L) and yield (0.29 g/g).

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