Biotechnological approches for green-based bioplastic production

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Abstract

Poly-3-hydroxybutyrate (PHB) is a biodegradable and biocompostable plastic produced by a wide variety of microorganisms as carbon reserve and electron sink. PHB presents physical and mechanical properties similar to fossil-based plastic, like polypropylene. Thanks to these features, PHB became a valuable alternative to plastic in several sectors, from packaging to medical applications. Nowadays, its production at industrial scale is slightly diffused, and the research is mainly focused on the optimization of its production process, and on the discovery of new and more sustainable fermentation routes.

In this context, this work dealt with the investigation on three main problems related with PHB fermentation: the application of a waste, such as carbon monoxide, as feedstock for the fermentation; the optimization of PHB production by a variation of fermentation parameters; the implementation of a greener and more cost-effective extraction protocols for the biopolymer. To achieve these goals, specific bacterial strains were selected and studied in this investigation.

Firstly, the fermentation conditions were developed to allow the growth of the wild type strain of *Rhodospirillum rubrum*, studying the influence of carbon monoxide concentration on the bacterial growth in 30 mL of culture. In the absence of light and sole CO as the primary carbon source, this strain needed a preliminary acclimation step, which strongly influenced the growth trend of subsequent fermentation. Because of the low solubility of the gaseous substrate, a pressurized fermentation was set in a dedicated 2L reactor. And, bacterial growth and PHB production at ambient and overpressure conditions (up to 8 atm) were compared. The application of overpressure did not change the biomass growth, but a consistent increase of PHB accumulation inside the cell was registered, passing from 13% in the absence of pressure, to 37% in the pressurized system.

On the other side, the strain *Azotobacter vinelandii* OP, characterized by a high specific growth rate and a higher percentage of PHB accumulation (up to 80%), was exploited for the optimization of PHB productivity based on the variation of the gas transfer rate in a fed-batch configuration. The investigation highlighted a strong correlation between the gas transfer rate, the specific growth rate and the PHB accumulation. Therefore, the optimal agitation conditions, matching the highest volumetric productivity, were defined at a gas mass transfer constant (k_La) of 100 h^{-1}.

Finally, it was investigated a protocol for the extraction of the biopolymer from the cell. Using a genetically modified *Escherichia coli* as bacterial strain, wet and dry not-pre-treated biomass were exposed to a green-based solvent (*i.e.* dimethyl carbonate) to define the PHB extraction yield and the biopolymer purity. From this case-study, a highly pure PHB was extracted with a 70% of extraction yield, proving that this green-based approach could be a real alternative to the standard PHB extraction procedures.