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Evaluation of by-products from the biodiesel industry as fermentation feedstock for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) production by *Cupriavidus necator*

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HIGHLIGHTS

- Valorisation of biodiesel industry by-products for PHA production.
- Replacement of commercial carbon sources and nutrient supplements.
- Replacement of precursors for co-polymer production.
- Influence of salt impurities concentration on PHA production.
- Analysis of thermophysical properties of the produced PHAs.

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ABSTRACT

Utilization of by-products from oilseed-based biodiesel production (crude glycerol, rapeseed meal hydrolysates) for microbial polyhydroxyalkanoate (PHA) production could lead to the replacement of expensive carbon sources, nutrient supplements and precursors for co-polymer production. Batch fermentations in shake flasks with varying amounts of free amino nitrogen led to the production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) with a 2.8–8% 3HV content. Fed-batch fermentations in shake flasks led to the production of 10.9 g/L P(3HB-*co*-3HV) and a 55.6% P(3HB-*co*-3HV) content. NaCl concentrations between 2 and 6 g/L gradually became inhibitory to bacterial growth and PHA formation, whereas in the case of K_2SO_4, the inhibitory effect was observed only at concentrations higher than 20 g/L. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and nuclear magnetic resonance (^{13}C NMR) demonstrated that the incorporation of 3HV into the obtained P(3HB-*co*-3HV) lowered glass transition temperature, crystallinity and melting point as compared to polyhydroxybutyrate. Integrating PHA production in existing oilseed-based biodiesel plants could enhance the viability and sustainability of this first generation biorefinery.

1. Introduction

Most processes for biodiesel production generate significant quantities of by-products. For instance, the utilization of oilseeds as raw materials leads to the production of a protein-rich oilseed meal and a crude glycerol stream. Valorisation of oilseed meals and crude glycerol into various products including chemicals, biodegradable polymers, value-added ingredients (e.g. extracts with antioxidant properties), food and feed would improve the economics of biodiesel production. Crude glycerol is a platform chemical that could be converted into different chemicals through chemical synthesis or fermentation (Koutinas et al., 2007).

Polyhydroxyalkanoates (PHAs) are a family of biodegradable polymers produced as intracellular energy-reserve granules during fermentation by more than 300 microorganisms including *Cupriavidus necator* (Lee, 1996; Choi et al., 1998). The microbial production of PHAs by *C. necator* is mainly based on the limitation of a nutritional element such as N, P, Mg, K, O, or S in the presence of an abundant source of carbon. PHAs can be used as substitutes

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