Bioresource Technology 118 (2012) 272–280

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Glycerine and levulinic acid: Renewable co-substrates for the fermentative synthesis of short-chain poly(hydroxyalkanoate) biopolymers ☆

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HIGHLIGHTS

- Combined glycerine and levulinic acid result in short-chain (sc) PHA synthesis.
- Sc-PHA properties were dictated by substrate ratio and fermentation duration.
- Reduced PHA molar masses based on glycerine-based chain termination.
- PHA compositions ranged from poly-3-hydroxybutyrate to poly-3-hydroxyvalerate.

ARTICLE INFO

Article history:
Received 9 March 2012
Received in revised form 14 May 2012
Accepted 19 May 2012
Available online 26 May 2012

Keywords:
Glycerine
Levulinic acid
Short-chain polyhydroxyalkanoate
Physical properties
Pseudomonas oleovorans

ABSTRACT

Glycerine (a biodiesel co-product) and levulinic acid (a pulp and paper co-product) were used as co-substrates for the fermentative synthesis of short-chain polyhydroxyalkanoate (sc-PHA) biopolymers with tunable monomer and molecular weight characteristics. *Pseudomonas oleovorans* NRRL B-14682 utilized glycerine alone to produce poly(3-hydroxybutyrate) (PHB). When levulinic acid was added to the media at shake-flask scale in concentrations ⩽0.6 wt.%, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB/V) copolymers were produced with 3-HV contents ranging from 37 to 97 mol%; a glycerine:levulinic acid ratio of 0.2%:0.8% (w/v) resulted in poly(3-hydroxyvalerate) (PHV). Ten-liter batch fermentations using glycerine:levulinic acid ratios of 1%:0, 0.75%:0.25%, 0.5%:0.5% and 0.25%:0.75% (w/v) resulted in PHB, P(73%-3HB-*co*-27%-3HV), P(30%-3HB-*co*-70%-3HV) and PHV with increasing number average molecular weights ($\times 10^3$ g/mol) of 328, 511, 728 and 1330, respectively, owing to glycerine-based chain termination. These results provide a novel means by which glycerine and levulinic acid can be used collectively to produce an array of distinct sc-PHA biopolymers.

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1. Introduction

Increased environmental concern has prompted the search for "green" substitutes for many petroleum-based materials. This search has led to the development of new uses for renewable feedstocks as precursors for many bio-based products. Generally however, the synthesis of bio-based materials requires a higher overhead than petroleum-based products. As such, the development of large-scale industrial applications for many bio-based materials has been impeded due to cost.

Poly(hydroxyalkanoates) (PHA) represent a family of structurally diverse bacterial polyesters that are synthesized as carbon and energy reserves by numerous bacterial species from many different carbon substrates. Synthesis generally occurs when a surplus of exogenous carbon is present and cellular growth is impeded by the lack of some other essential nutrient. Because of their structural variability, PHA biopolymers exhibit an array of material properties from rigid thermoplastics to amorphous elastomers. Poly(3-hydroxybutyrate) (PHB), the simplest and most well-known of the PHA biopolymers, has been compared to polypropylene (PP) and polyethylene (PE) with respect to its environmental impact and its material properties. Life cycle analysis (LCA) showed that PHB was more favorable than PP and PE in environmental impact by surpassing both in its effects on abiotic and ozone depletion, global warming, human and ecotoxicity, acidification and eutrophication (Harding et al., 2007). However, because of its highly crystalline nature, PHB is considered too rigid and

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0960-8524/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biortech.2012.05.092