Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Downstream separation of poly(hydroxyalkanoates) using crude enzyme consortia produced via solid state fermentation integrated in a biorefinery concept

Vasiliki Kachrimanidou[a], *Nikolaos Kopsahelis*[a,b,*], *Anestis Vlysidis*[a], *Seraphim Papanikolaou*[a], *Ioannis K. Kookos*[c], *Belén Monje Martínez*[d], *Maria Consuelo Escrig Rondán*[d], *Apostolis A. Koutinas*[a,*]

[a] *Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece*

[b] *Department of Food Technology, Technological Educational Institute (TEI) of Ionian Islands, Argostoli 28100, Kefalonia, Greece*

[c] *Department of Chemical Engineering, University of Patras, Rio 26504, Patras, Greece*

[d] *AIMPLAS, Instituto Technológico del Plástico, València Parc Tecnològic, Gustave Eiffel 4, 46980 Paterna, Valencia, Spain*

ARTICLE INFO

Article history:
Received 18 May 2016
Received in revised form 29 July 2016
Accepted 1 August 2016
Available online 9 August 2016

Keywords:
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
Cell disruption
Downstream processing
Integrated processing
Solid state fermentation
Sunflower meal

ABSTRACT

Crude enzyme produced via solid state fermentation using the fungal strain *Aspergillus oryzae* was used to lyse cells of *Cupriavidus necator*, enabling the recovery of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), P(3HB-*co*-12 mol% 3HV). A central composite design was employed in order to optimize the temperature and the pH value leading to the highest lysis of *C. necator* cells (88.9 ± 0.4%) and the optimal hydrolysis of protein contained in bacterial cells. Enzymatic lysis of bacterial cells was also carried out at the optimum temperature and uncontrolled pH value leading to P(3HB-*co*-12 mol% 3HV) recovery yield and purity of 98% and 96.7%, respectively. The bacterial cell lysate obtained after the separation of P(3HB-*co*-12 mol% 3HV) granules was evaluated as nutrient-rich supplement together with crude glycerol for the production of poly(3-hydroxybutyrate) in shake flask cultures. This novel downstream separation process could be integrated in a sunflower-based biorefinery reducing the cost of poly(hydroxyalkanoate) recovery and fermentation medium formulation.

© 2016 Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

1. Introduction

Under the frame of constructing a sustainable bio-based economy, the global manufacturing capacity of bio-based polymers is projected to increase three-fold from 5.7 million t in 2014 to 17 million t in 2020 with poly(hydroxyalkanoates) (PHA) expected to show the second most dynamic development (Aeschelmann and Carus, 2016). PHA are a family of biodegradable polyesters produced intracellularly by various bacterial and archaeal strains as energy and carbon reserve granules. PHA biosynthesis is most frequently triggered after the depletion of nutrients (e.g., nitrogen, phosphorus) from the fermentation medium, while there is still an excess carbon source. Industrial implementation of PHA production is hindered by the high cost of manufacture

* *Corresponding authors at*: Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece.
E-mail addresses: kopsahelis@upatras.gr (N. Kopsahelis), akoutinas@aua.gr (A.A. Koutinas).
http://dx.doi.org/10.1016/j.fbp.2016.08.002
0960-3085/© 2016 Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

which is mainly attributed to the fermentation media employed and the downstream separation and purification of PHA. The former bottleneck could be overcome by substituting the expensive and purified components used in the formulation of fermentation medium (e.g., yeast extract, pure glycerol) with nutrient rich supplements derived from renewable resources. For instance, crude glycerol has been widely employed for the production of PHA using either synthetic media (Cavalheiro et al., 2009; Hermann-Krauss et al., 2013) or crude nutrient supplements derived from renewable resources, such rapeseed meal (Garcia et al., 2013), sunflower meal (Kachrimanidou et al., 2014, 2015) and wine lees (Dimou et al., 2015). Crude nutrient supplements could be produced via enzymatic hydrolysis of renewable resources, providing a complete fermentation medium, including nitrogen, phosphorus and micronutrients, for the production of PHA or other metabolic products, such as 1,3-propanediol, microbial oil and bacterial cellulose (Chatzifragkou et al., 2014; Tsakona et al., 2014; Tsouko et al., 2015).

The second impediment on the industrial production of PHA relates to downstream separation of intracellular polyesters. Isolation and purification of PHA have been extensively studied regarding the development of environmentally benign and cost competitive processes for industrial implementation (Jacquel et al., 2008; Gumel et al., 2012; López-Abelairas et al., 2015). Solvent extraction of poly(3-hydroxybutyrate) (PHB) has been widely employed using various solvents, including non-chlorinated industrial solvents such as anisole, cyclohexanone and phenetole (Rosengart et al., 2015). PHA separation has also been achieved via digestion of non-PHA bacterial cell mass using chemical (using acids or alkalis) or enzymatic treatment (Gumel et al., 2012; López-Abelairas et al., 2015). Mechanical disruption of non-PHA bacterial cell mass can also be combined with subsequent treatment using surfactants (e.g., anionic sodium dodecyl sulfate) or chemicals (Jacquel et al., 2008; Gumel et al., 2012). PHA recovery may also be carried out with processes involving supercritical fluid extraction, air classification and dissolved-air flotation (Jacquel et al., 2008; Gumel et al., 2012; Koller et al., 2013). Despite the high polymer purity accomplished by chemical methods, the environmental benignness of such downstream separation methods is low due to the large volumes of solvents and surfactants required.

Enzymatic cell lysis has many advantages including low energy requirements, biological specificity, mild operating conditions and low capital investment (Harrison, 1991). However, the high cost of enzyme production is a major disadvantage regarding their industrial implementation. In the case of PHA recovery, commercial proteolytic enzymes have been mainly applied (Kapritchkoff et al., 2006; Yasotha et al., 2006; Holmes and Lin, 1990). Enzymatic digestion of non-PHA cell mass has been previously demonstrated as the sole method of purification or coupled with the utilization of surfactants to decolorize and deodorize the polymer or to remove cell debris (Suzuki et al., 2008; Lakshman and Shamala, 2006; Horowitz and Brennan, 2010). Holmes and Lim (1990) reported the utilization of proteolytic enzymes and surfactant treatment to disintegrate the bacterial mass of *Alcaligenes eutrophus* NCIB 11599 for PHB separation. The potential valorization of hydrolyzed cell mass was also proposed as a potential approach for the re-utilization of nutrients released during enzymatic lysis (Holmes and Lin, 1990). Cost competitive production of enzymes could be achieved through solid state fermentation (SSF) of fungal strains using diversified agro-industrial waste and by-product streams (Melikoglu et al., 2013; Diaz et al., 2013). Fungal strains of *Aspergillus oryzae* produce various enzymes including protease, phosphatase, pectinase and lipase (Toida et al., 1998; Kachrimanidou et al., 2013; Heerd et al., 2012). The *A. oryzae* strain used in this study produces mainly proteases among various enzymes the synergistic action of which could lead to hydrolysis of bacterial cell mass leading to the recovery of PHA.

The cell wall of gram-negative bacteria is composed of a peptidoglycan layer surrounded by an outer membrane, consisting mainly of proteins, phospholipids, lipoproteins and lipopolysaccharides (da Silva et al., 2012). Gram-negative bacteria are resistant to enzymatic lysis by lytic enzymes that hydrolyze only the peptidoglycan layer, such as lysozymes and murein hydrolases. Therefore, the effective lysis of the cell wall of gram-negative bacteria can be accomplished by the combination of various enzymes, such as amidase, lipase and protease. Filamentous fungi can produce a combination of enzymes required for the lysis of the cell wall of gram-negative bacteria (da Silva et al., 2012). Tsakona et al. (2016) showed that crude enzymes preparation produced via solid state fermentation of *Aspergillus awamori* could be used for the lysis of oleaginous yeast cells leading to the release of microbial oil that is originally produced as an intracellular product.

Kachrimanidou et al. (2014, 2015) have employed biodiesel industry by-products, namely sunflower meal (SFM) and crude glycerol, for the development of a biorefinery concept leading to the production of antioxidants, protein isolate and crude hydrolysates. The latter can be used as fermentation supplements for the production of either PHB or poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), P(3HB-*co*-3HV), after the addition of levulinic acid as precursor. The nutrient rich hydrolysates were produced either from SFM alone or from various fractions derived via SFM fractionation. A two-stage bioprocess involving solid state fermentation carried out with *A. oryzae* for the production of crude enzymes was followed by enzymatic hydrolysis of crude SFM or various fractions.

The aim of the present study is to demonstrate the ability to recover the P(3HB-*co*-3HV) produced during fermentation of crude glycerol, SFM hydrolysates and levulinic acid through enzymatic cell lysis using the crude enzyme preparation produced during solid state fermentation by *A. oryzae*. In this way, the enzymes produced via SSF could be employed for downstream separation of P(3HB-*co*-3HV), besides the production of nutrient-rich hydrolysates for the fermentation stage. A central composite design was applied in order to optimize the temperature and pH value during enzymatic cell lysis. After the separation of P(3HB-*co*-3HV), the cell lysate was recycled as nutrient-rich supplement together with crude glycerol for the production of PHB, enhancing the sustainability of the proposed biorefinery.

2. Materials and methods

2.1. Microorganisms and raw materials

An industrial strain of *A. oryzae*, provided by Professor Colin Webb (University of Manchester, UK) was utilized in solid state fermentations for the production of crude enzymes. The strain was maintained on slopes containing 30 g L^{-1} SFM, 20 g L^{-1} wheat bran and 20 g L^{-1} agar (Sigma–Aldrich) according to the protocol described by Kachrimanidou et al. (2013). Inoculation

Download English Version:

https://daneshyari.com/en/article/6451750

Download Persian Version:

https://daneshyari.com/article/6451750

Daneshyari.com