International Journal of Biological Macromolecules 112 (2018) 273–283

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/ijbiomac

Comparison of yields and properties of microbial polyhydroxyalkanoates generated from waste glycerol based substrates

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ARTICLE INFO

Article history:
Received 17 November 2017
Received in revised form 22 January 2018
Accepted 26 January 2018
Available online 31 January 2018

Keywords:
Waste glycerol
Polyhydroxyalkanoates
Thermal properties, mechanical properties

ABSTRACT

The accumulation efficiency and the properties of polyhydroxyalkanoates (PHAs) produced from acidified waste glycerol (AWG) and its derivatives via an enriched microbial consortium derived from soil, were investigated in this study. AWG consisted mainly from short chain fatty acids, 1,3 propanediol and residual glycerol, which were also evaluated individually as substrates. Accumulation capacity and yields were estimated after solvent extraction and purification and PHAs were further analyzed in terms of their chemical structure, thermal properties, molecular masses and mechanical properties. The lowest accumulation capacity was noticed for non-acidified waste glycerol as carbon source which led to the generation of P(3HB), whereas for the other carbon sources *co*-polymers of 3HB with 3HV or 3HHx were produced. Average molecular mass weights were quite high in all cases reaching ~1.8×10^6 Da. The thermal properties and the mechanical behavior of PHAs were shown to be highly affected by their monomeric composition, whereas it was also concluded that DSC and DMA results were in good agreement.

1. Introduction

In the recent years a lot of research has focused on the production of biodegradable bioplastics from renewable resources. Among them great interest has been expressed in the production of polyhydroxyalkanoates (PHAs), which are biological linear polyesters, accumulated intracellularly by various microbial species as carbon and energy reserves. PHAs have similar properties to petroleum-derived synthetic polymers, such as PVC and PPT, but they are also biodegradable, biocompatible, water resistant, and impermeable to oxygen. In this context, PHAs can be used in a variety of disposable packaging goods, finding also a high-value application in both medicine and the pharmaceutical industry [1,2].

Although an expanding interest has been shown during the previous decades for the commercialization of biobased biodegradable polymers, there are still different issues that have to be address in order PHAs to become antagonistic in the market. Indeed, while the market price of fossils bioplastics is approximately 1000–1500 $/MT, the price for biobased plastics varies from 4000 $/MT to as high as 15,000 $/MT for materials such as PHAs [3]. Both upstream and downstream processes seem to contribute equally to the high cost of PHAs production and efforts have been focused on findings ways for the reduction of them. In general there are two basic approaches for the recovery of PHAs from the microbial biomass i.e. the disruption of the microbial cell in order for the intracellular PHAs granules to be released from the biomass and the direct recovery from the cells via extraction using organic solvents while the recovered products has to be further purified before use. The choice of the recovery and purification methodology however cannot be based solely on the final cost, but also on the degree of purity and the conservation of the molecular weigh of the recovered final product while environmentally impact of each methodology needs to be taken into account [4]. Another important hurdle to the commercialization of such PHA producing bioprocesses is the cost of the chosen carbon source, which amounts up to 50% of the total production cost. As such, different types of wastes and residues (zero-cost substrates), have been so far examined as potential carbon sources for PHAs production [5], using either pure microbial cultures (PMCs) or mixed microbial cultures (MMCs) [6–11]. MMCs may be acclimated and enriched using nutrient limitation under periodic feeding [12–15], whereas it is also common to include a first step of acidification of the waste prior to PHAs production [9,16,17]. For high PHA productivity, generally fed-batch or sequential-batch fermentations are used, via which cell growth and PHA formation can be controlled under different nutrient limitation conditions [18].

The recent rapid growth of the biodiesel industry has generated a significant amount of glycerol (Waste glycerol (WG), also called crude glycerol) as a byproduct. WG may be used as feedstock for the production of

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https://doi.org/10.1016/j.ijbiomac.2018.01.175
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various high added-value chemicals and biofuels, in a biorefinery concept and different schemes have been so far proposed, based on either biological processes alone or on the combination of biological and physicochemical ones [19–21]. Among other alternatives, the use of WG as carbon source for the production of bioplastics via bacterial fermentation has been proposed, given its ubiquity, low price, and high degree of reduction. It has been observed, however, that the productivity and yield of PHAs from glycerol might be significantly lower compared to that from other carbon sources [22,23]. Moreover, glycerol as a chemical agent can function as the terminal end-group for PHB biosynthesis during the long exposure of cells to glycerol in fermentation, resulting in a reduction of the average molecular weight of the polymers [24].

In order to overcome such limitations, in the framework of the present study the effect of an initial acidification step of the WG prior to its bioconversion towards PHAs was investigated. Based on the initial acidification of WG an integrated multiple steps approach WG valorisation concept can be proposed, according to which, different high added-value bioproducts, such as biofuels, platform chemicals, bioplastics and fertilizer can be generated.

The overall concept is illustrated in Fig. 1. The first step of the process is the direct use of WG as substrate for the simultaneous production of biohydrogen, short chain fatty acids (SCFAs) and 1,3 propanediol (PDO) via its anaerobic fermentation with mixed acidogenic consortia in a continuous column bioreactor as described by Dounavis et al. [25]. The liquid effluent or this first 'acidification' step is an aqueous solution containing different metabolic products and non-consumed glycerol and a particulate fraction i.e. the microbial biomass. Two different approaches are considered for the subsequent valorization of the acidification effluents. In the first one, the liquid effluent may be directly fed to an anaerobic digester aiming at the recovery of energy in the form of methane as described in Dounavis et al. [26]. The digestate has almost zero dissolved chemical oxygen demand (d-COD) and its solid fraction can be further used for the production of compost [27]. In the second approach, the acidification effluents may be clarified via centrifugation, in order to remove the microbial biomass and the liquid fraction, called in this study acidified WG (AWG), may be used as carbon source for the production of PHAs. The fermentation products that are generated from the acidification of glycerol can act as the precursors of different types of PHAs including not only P(3HB), but also higher chain length PHAs with advanced properties. Thus, in this approach the valorization of WG is achieved via the recovery of energy in the form of H_2 (energy carrier) in a first acidification step, followed by bioconversion of the residual carbon remaining in the effluent towards PHAs. Such a two-stage valorization approach has been followed for the valorization of other wastes, such as olive-mill wastewater [13] and *Calophyllum inophyllum* oil cake [28].

The aim of the present study was to evaluate the type, yields and properties of the PHAs generated from AWG, allowing as such the assessment of the viability of the second WG valorization approach. Since AWG is actually a mixture of various chemical compounds, the production efficiency of PHAs generated from different mixtures of the key identified compounds in the acidification effluent was also tested. Subsequently, the chemical structure, thermal properties and molecular masses, as well as the mechanical properties -via both static and dynamic methods- of the different produced PHAs were assessed and compared. Up to our knowledge it is the first time that both physicochemical and mechanical properties of PHAs generated via mixed cultures from WG based substrates are assessed, providing thus important information for its valorization.

2. Materials and methods

2.1. Enrichment of microbial culture for PHA production

An enriched MMC derived from soil was used in all experiments of the present study. For the enrichment, a 10% *w*/*v* soil suspension (clay soil, sample from Platani, Rio, Greece; 38° 18′ N / 21° 49′ E) was prepared with distilled water and was kept at ambient temperature under constant agitation (250–300 rpm) for 2 h. Subsequently, it was heated to 70 °C for 10 min, cooled, and the suspended solids were allowed to settle. The supernatant was filtered via a 0.5 mm sieve and was supplemented with both carbon and nitrogen sources in order to allow initial non- selective microbial growth. The composition of the medium used was: acetic acid, 1 $g{\cdot}L^{-1}$; butyric acid, 1 $g{\cdot}L^{-1}$; propionic acid, 1 $g{\cdot}L^{-1}$; $(NH_4)_2SO_4$, 1 $g{\cdot}L^{-1}$; K_2HPO_4, 2.5 $g{\cdot}L^{-1}$; KH_2PO_4; 1.5 $g{\cdot}L^{-1}$, $MgSO_4 \times 7H_2O$, 0.2 $g{\cdot}L^{-1}$; $CaCl_2 \times 2H_2O$, 0.05 $g{\cdot}L^{-1}$; trace elements solution, 1 $mL{\cdot}L^{-1}$. The trace elements solution was prepared separately as follows: 10 $mg{\cdot}L^{-1}$ $CuSO_4x2H_2O$, 2 $g{\cdot}L^{-1}$ $FeSO_4 \times 7H_2O$, 30 $mg{\cdot}L^{-1}$ $NaMoO_4 \times 2H_2O$, 20 $mg{\cdot}L^{-1}$ $NiCl_2 \times 6H_2O$, 1 $g{\cdot}L^{-1}$ $ZnSO_4 \times 7H_2O$, 0.2 $g{\cdot}L^{-1}$ $CoCl_2 \times 6H_2O$, 0.3 $g{\cdot}L^{-1}$ KI, 0.3 $g{\cdot}L^{-1}$ H_3BO_3, 30 $mg{\cdot}L^{-1}$ $MnCl_2 \times 4H_2O$ and 2.5 $g{\cdot}L^{-1}$ EDTA. Subsequently, the enhancement of the initial culture with PHAs producers was performed, by subjecting it to sequential nitrogen/carbon limitation as previously reported by Ntaikou et al. [12]. As such the mixed microbial consortium

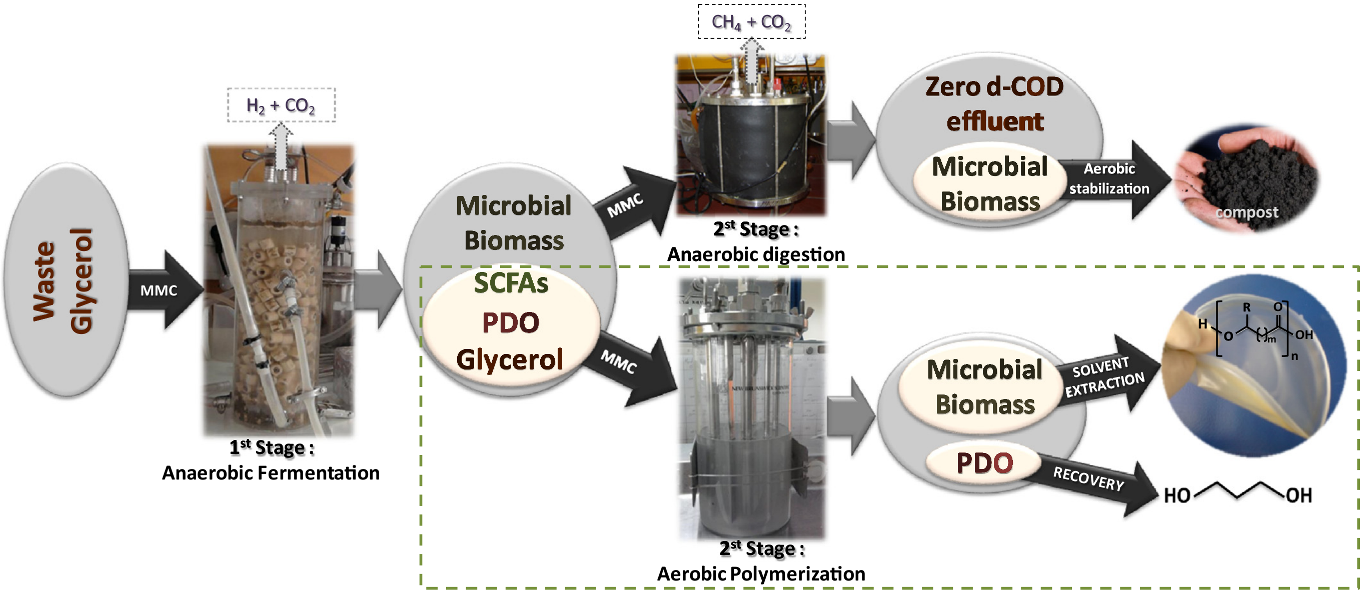

Fig. 1. Schematic illustration of the concept proposed in this study for the valorization of WG via microbiological processes.