New Biotechnology • Volume 33, Number 1 • January 2016

Valorization of fatty acids-containing wastes and byproducts into short- and medium-chain length polyhydroxyalkanoates

Madalena V. Cruz[1], Filomena Freitas[1], Alexandre Paiva[2], Francisca Mano[2], Madalena Dionísio[2], Ana Maria Ramos[2] and Maria A.M. Reis[1]

[1] UCIBIO-REQUIMTE, Chemistry Department, FCT/Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[2] LAQV-REQUIMTE, Chemistry Department, FCT/Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

Olive oil distillate (OOD), biodiesel fatty acids-byproduct (FAB) and used cooking oil (UCO) were tested as inexpensive carbon sources for the production of polyhydroxyalkanoates (PHA) with different composition using twelve bacterial strains. OOD and FAB were exploited for the first time as alternative substrates for PHA production. UCO, OOD and FAB were used by *Cupriavidus necator* and *Pseudomonas oleovorans* to synthesize the homopolymer poly-3-hydroxybutyrate, while *Pseudomonas resinovorans* and *Pseudomonas citronellolis* produced mcl-PHA polymers mainly composed of hydroxyoctanoate and hydroxydecanoate monomers. The highest polymer content in the biomass was obtained for *C. necator* (62 wt.%) cultivated on OOD. Relatively high mcl-PHA content (28–31 wt.%) was reached by *P. resinovorans* cultivated in OOD. This study shows, for the first time, that OOD is a promising substrate for PHA production since it gives high polymer yields and allows for the synthesis of different polymers (scl- or mcl-PHA) by selection of the adequate strains.

Introduction

Polyhydroxyalkanoates (PHA) are biodegradable and biocompatible polyesters that occur as intracellular carbon and energy reserves in many bacteria. Depending on the selected bacterial species and on the carbon source used during the fermentation process, different types of polymers can be produced, namely, short-chain length PHA (scl-PHA), mainly composed by 3-hydroxy fatty acids of 3–5 carbon units, and medium-chain length PHA (mcl-PHA) that contain units higher than 5 carbons in length [1]. The different polymer composition influences the physical–chemical, thermal and mechanical properties. Most scl-PHAs are very rigid and brittle biothermoplastics, due to their high crystallinity (50–70%), while mcl-PHAs are more elastic and/or viscous materials, characterized by low crystallinity degrees, glass transition temperatures and melting temperatures [2,3].

PHAs are commonly produced from simple carbon sources, such as sugars (e.g. glucose or sucrose). However, the high price of those feedstocks accounts for up to 50% of the overall PHA production costs [1]. Therefore, in the past years, cheaper or inexpensive carbon sources, such as vegetable oils [4–6], have been studied as possible candidates for PHA production. Vegetable oils are very attractive, since the theoretical PHA yield can be as high as 1 g g^{-1} [6]. Yields around 0.6–0.8 g g^{-1} were reported for oil substrates, which are higher than those for sugars (0.3–0.4 g g^{-1}) [7]. Due to their composition in medium and long chain fatty acids units, oil-containing substrates can act as precursors for different types of PHA, with specific properties leading to novel possible applications of the final product.

Used cooking oil (UCO) is a food industry waste mainly composed of triglycerides, containing long chain fatty acids (e.g. palmitic, oleic and linoleic acids) with saturated and/or unsaturated bonds. Although UCO is usually valorized by its conversion into biodiesel, there is a surplus of this waste that can be efficiently

---

Corresponding author: Freitas, F. (a4406@fct.unl.pt)

http://dx.doi.org/10.1016/j.nbt.2015.05.005

1871-6784/© 2015 Elsevier B.V. All rights reserved.

converted into PHA, with the advantage of giving higher polymer yields compared to those obtained from sugars.

Some PHA storing bacteria are not capable to convert complex triglyceride structures into simpler units composed by fatty acids, since they do not have a strong lipolytic activity [8]. For these cases, prior hydrolysis to yield free fatty acids (FFA) or saponification of the triglyceride structures is required [4], which increase the overall production costs. Moreover, FFAs are more water-miscible than the hydrophobic structures of triglycerides, thus facilitating homogenization of the cultivation broth and facilitating transport phenomena. For this reason, FFA-enriched wastes and/or byproducts should also be considered as potential candidates for PHA production.

Olive oil distillate (OOD), a low market value (0.24–0.39 US$/Kg) by-product from the olive oil refining industry representing 0.05–0.1% of the total processed oil, is mainly composed by FFA (>50 wt.%) with smaller fractions of other valuable compounds (e.g. squalene, tocopherols, sterols esters, etc.) [9]. Typically, OOD is mixed with other refining by-products in the neutralization step of the refining process [9]. Though some authors have been interested in the recovery of the valuable compounds from OOD, it was still not exploited as a carbon source for PHA production. Fatty acids by-product from the biodiesel industry (FAB) is generated during glycerol purification and it contains large amounts of soap FFA and minor amounts of other unused reactants [10]. The potential of this byproduct for PHA production was still not investigated.

Among the known PHA-accumulating bacterial strains, *Cupriavidus necator* [5,11] and several *Pseudomonas* species (e.g. *P. resinovorans* [1], including *P. citronellolis* [8,12] and *P. oleovorans* [4]) have been reported as capable to convert fatty acids-containing substrates (e.g. fish oil byproduct, tallow FFA, saponified *Jatropha curcas* oil, low quality biodiesel, etc.) into PHA.

The goal of this study was to valorize different fatty acids-containing wastes and byproducts (UCO, OOD and FAB) by their conversion into different types of PHA (scl- and mcl-PHA) using several PHA-accumulating strains belonging to the Genera (*Cupriavidus*, *Pseudomonas*, *Comamonas* and *Azotobacter*). Significant relevance was given to the characterization of the carbon source to better correlate their composition with bacterial strain metabolic activity and performance for PHA production. The final product properties (composition, molecular mass distribution and thermal properties) were also accessed.

Material and methods

Characterization of the fatty acids-containing substrates

UCO was supplied by the University's cantina; OOD was supplied by Sovena Group SA, Portugal, which is a vegetable oil-refining and packaging industry; fatty-acids methyl esters (FAME), FAB and neutralized soybean oil (SOY) were supplied by SGC Energia SA, Portugal. SOY (high triglyceride content oil) and FAME (transesterified oil, enriched in methyl esters) were tested as control substrates. SOY was a non-edible oil resulting from the neutralization step of the biodiesel production process.

The acylglycerides content, namely, mono-, di- and triglycerides of the five substrates were determined by gas chromatography (GC), according to the European norm EN 14105. Squalene content was determined as described by Rocha *et al.* [13]. All the data was processed with software Chrom-Card.

Total free FFA content was quantified by titration, according to the AOCS official method Ca 5a-40, using Eq. (1):

$$FFA_{as\,oleic\,acid}, \% = \frac{V_{NaOH} \times C_{NaOH} \times 28.2}{m_i} \tag{1}$$

where V_{NaOH} (mL) is the volume of sodium hydroxide titrated, C_{NaOH} (g mol^{-1}) is the concentration of the alkali solution and m_i (g) the weighted mass of the substrate.

The fatty acid composition of the oils was determined by direct transesterification of the lipids to the corresponding methyl esters (FAME), according to a modified method [14]. Briefly, 25 mg of the fatty acids-containing substrate were mixed with 2 mL of methanol containing 5% (v/v) of acetyl chloride and heated at 80 °C, for 1 h. Afterwards, the samples were cooled to room temperature and 1 mL of hexane and 1 mL of deionized water were added. The samples were stirred in the vortex for 30 s. FAME quantification and identification was performed by GC according to the EN14103. Peak identification was carried out using known standards (FAME mixture). Methylheptadecanoate (10 mg mL^{-1}) was used as internal standard. All analyses were performed in duplicate.

PHA production

Microorganisms and media

Pseudomonas oleovorans NRRL B-14682, NRRL B-778, NRRL B-14683 and NRRL B-3429, *P. citronellolis* NRRL B-2504, *P. resinovorans* NRRL B-2649, *P. stutzeri* NRRL B-775, *C. necator* NRRL B-4383, *Comamonas testosteroni* NRRL B-2611 and *Azotobacter vinelandii* NRRL B-14641 were kindly offered by the Agricultural Research Service Culture (NRRL) Collection, USA. *C. necator* DSM 428 was purchased from the German Collection of Microorganisms and Cell cultures (DSMZ), Germany, and *Pseudomonas corrugata* 388 was kindly offered by Dr. Daniel Solaiman from the United States Department of Agriculture (USDA). All bacterial strains were kept in frozen stocks (−80 °C) in LB (Luria-Bertani) medium (pH = 6.8), with the following composition (per liter): bacto-tryptone, 10 g; yeast extract, 5 g; sodium chloride, 10 g. Glycerol (20%, v/v) was added as a cryoprotectant. Reactivation from the stock culture was performed by inoculation in solid LB medium (15 g L^{-1} of agar). Inocula for batch cultivations were prepared by inoculation of a single colony into liquid LB medium and incubation in an orbital shaker, at 30 °C and 200 rpm, for 24 h. Afterwards, the culture was transferred into mineral medium with the composition described by Freitas *et al.* [15], supplemented with the fatty acids-containing substrate (20 g L^{-1}).

Screening for oil-utilizing and PHA-producing bacteria

The twelve bacterial strains were tested by cultivation on mineral medium supplemented with each of the fatty acids-containing substrates (UCO, OOD and FAB) (20 g L^{-1}). SOY and FAME were used as control experiments for cultivations in UCO and FAB, respectively. The experiments were performed in SeptaVent^{TM} HTS (50 mL) flasks, at 30 °C and 200 rpm, with an initial pH-value of 6.8 ± 0.2. All experiments took 96 h. A 10% (v/v) inoculum was used in each experiment. Daily samples were taken for evaluation of the cell growth by measurement of the broth's optical density (at 600 nm). The cells were visualized under the optical microscope (Olympus BX51), in phase contrast, and polymer accumulation was qualitatively evaluated by Nile blue A staining, as

Download English Version:

https://daneshyari.com/en/article/33053

Download Persian Version:

https://daneshyari.com/article/33053

Daneshyari.com