chemistry proceedings

MDPI

Proceeding Paper

Studies on *mcl*-Polyhydroxyalkanoates Using Different Carbon Sources for New Biomedical Materials †

Dana-Maria Miu [1,2,*], Mariana-Gratiela Vladu [2,3] and Sorin-Ion Jinga [1]

1 Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, 1-7 Gh. Polizu Street, 011061 Bucharest, Romania; sorinionjinga@yahoo.com
2 National Institute for Chemical - Pharmaceutical Research and Development – ICCF Bucharest, 112 Vitan Ave, 031299 Bucharest, Romania; marianagratielavladu@gmail.com
3 Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Boulevard, District 1, 011464 Bucharest, Romania
* Correspondence: dana.miu92@gmail.com
† Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: https://ecsoc-24.sciforum.net/.

Abstract: Polyhydroxyalkanoates (PHAs) are microbial homo- and copolymers of [R]-β-hydroxyalkanoic acids, produced by a wide variety of bacteria as an intracellular carbon and energy reserve. To obtain *mcl*-PHAs of microbial origin, we used a *Pseudomonas* spp. strain (from the National Institute for Chemical-Pharmaceutical Research and Development (ICCF) culture collection of micro-organisms), by varying the carbon sources and the precursors. In this work, assays were performed with fermentation media seeded with inoculum cultures of strain *Pseudomonas putida* in a proportion of 10%. The influence on strain development and *mcl*-PHA production of carbon sources consisting in C6, C7, C8 and C9 fatty acids (as polymers precursors) was analyzed. Due to their properties, similar to conventional plastics and their biodegradability, PHAs are suitable for many applications and for biomedical materials useful in surgical sutures, tissue engineering and drugs carriers, leading us to deepen the study of obtaining micro/nanofibers by the electrospinning method.

Keywords: polyhydroxyalkanoates; bioprocess; biomaterials; electrospinning

Citation: Miu, D.-M.; Vladu, M.-G.; Jinga, S.-I. Studies on *mcl*-Polyhydroxyalkanoates Using Different Carbon Sources for New Biomedical Materials. *Chem. Proc.* **2021**, *3*, 143. https://doi.org/10.3390/ecsoc-24-08429

Academic Editor: Aggie Zheng

Published: 14 November 2020

1. Introduction

Polyhydroxyalkanoates (PHA) are microbial homo- and copolymers of [R]-β-hydroxyalkanoic acids, are produced by a wide variety of bacteria as an intracellular carbon and energy reserve [1,2]. The factors that affect the growth of the microorganism and implicitly the PHAs production, depend very much on the composition of the medium, and are as follows: the concentration and the type of the carbon source, the amount of nitrogen and phosphorus source. Other factors, also important, are pH, temperature, oxygen concentration, and the system of cultivation and they can influence the conversion of the substrate and the content of PHA in the cells [2–4]. Depending on the number of carbon atoms, contained by the monomers units, PHAs isolated can be classified as follows: (i) short chain length (*scl*) PHAs—3 to 5 carbon atoms/monomer, (ii) medium chain length (*mcl*) PHAs—6–14 carbon atoms/monomer, and *scl*-co-*mcl* with repeat-unit monomers containing 3–14 carbon atoms [2]. Many studies confirmed that *mcl*-PHA type is much more flexible and resistant than *scl*-PHAs [5,6].

Due to the fact that they have properties similar to plastics obtained from petroleum, but especially due to the fact that they are biodegradable, PHA can be an alternative to synthetic polymers [7]. These are promising materials due to their useful characteristics: thermoplastic and elastomeric properties, biodegradability, biocompatibility and nontoxicity. Consequently, they are good candidates for various applications in industry (replacements for petroleum-derived plastics, packaging industry, laminate papers and

cardboards), fine chemical industry (starting materials for the synthesis of antibiotics and other fine chemicals) or medicine (scaffolds for bone tissue engineering, drug delivery system) [8–11].

In this paper, we studied the optimal concentration of fatty acids to obtain new biomaterials used in medical domain.

2. Materials and Methods

The ingredients and the reagents used in experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck (Kenilworth, NJ, USA), and Larodan (Solna, Sweden).

For PHA production *Pseudomonas putida* ICCF 391 was used. The stock culture was grown at 29 ± 1 °C and periodically transferred on fresh M44 (cDSMZ424) agarized medium. During the research, the stock cultures were kept at 5 °C in the refrigerator. Pre-inoculum medium (M44) contained (g/L): yeast extract 10, peptone 10, glycerol 50, agar 20. The cell culture from the pre-inoculum medium was taken in 2 mL of distilled water and passed into the inoculum medium (100 mL), whose composition was (g/L): glucose 10, corn extract 15, KH_2PO_4 10, NaCl 10, $MgSO_4$ 0.5. The inoculum culture, developed 24 h at 30 °C on shaker (220 rpm), was used in a proportion of 10% for the inoculation of the fermentation medium. The medium (250 mL/flask) used to produce PHAs contained (g/L): $NaNH_4HPO_4 \cdot 4H_2O$ 3.5, K_2HPO_4 7.5, KH_2PO_4 3.7, and was periodically supplemented (at 0 and 24 h) with fatty acids investigated (C6, C7, C8, C9), in different combinations, whose amount varied in the range 15.23–16.7 g/L. The experiments were performed to make a comparison between the degree of conversion of fatty acids and the composition of the polymers obtained, when a single precursor (C8, C9) or combinations of precursors (C8–C9, C6–C8, C7–C9) were added to the bioprocess medium. At the time of inoculation, the bioprocess medium was supplemented with two solutions containing trace elements (1.0 mL/L from each solution) prepared and sterilized separately [3].

After dissolving the ingredients in distilled water and adjusting the pH to 6.8–7.00, the media were sterilized for 30 min at 115 °C.

The bioprocesses were conducted for 48 h, at 30 °C, and 220 rpm, and the optical density (OD) of the culture was measured periodically (at λ = 550 nm, 1:25 dilution) with a spectrophotometer (UV-VIS, Jasco V-Able 630). After centrifugation and processing of the bioprocess media, the amount of dry biomass and the amount of polyhydroxyalkanoates obtained were determined.

The biomass (obtained after centrifugation of the medium) was treated with methanol and then dryied under vacuum. The *mcl*-PHAs were extracted from biomass by acetone Soxhlet extraction method (biomass: acetone ratio was 1:20). The next step consisted in the concentration of the extract obtained and the precipitation of the *mcl*-PHA with cooled (in refrigerator) methanol (1:10 concentrated extract: methanol). The precipitated polymer has been dissolved in chloroform, the chloroform was evaporated, the polymer was left to dry and after that, weighed [2].

In order to determine the monomer composition of the obtained polymers, acid methanolysis of these polymers was performed, which resulted in obtaining a mixture of methyl esters, further identified chromatographically, based on methyl esters standards C6-C11, using an HP 5 column (5% phenyl-methyl-polysiloxane) [2,12]. After chromatographic identification of the monomers, their purity degree was calculated to determine the degree of conversion of the substrate consisting of fatty acids provided as a carbon source.

3. Results and Discussion

The amount of precursors, the manner of supplementation and their type, as well as the results obtained at the end of the bioprocesses, performed in order to obtain PHA, are presented in Table 1.

Table 1. The addition of the precursors and the values of the parameters obtained at the end of the fermentation.

Samples	Precursors Added (g/L)		Parameters Values		
	0 h	24 h	pH	OD [1]	DCW [2] (g/L)
P5	8.35 C8	8.35 C8	7.32	0.425	3.370
P7	8.35 C9	8.35 C9	7.35	0.241	1.619
P13	8.35 C8	8.35 C9	7.16	0.353	1.619
P14	8.35 C9	8.35 C8	7.15	0.422	1.648
P17	8.35 C9	8.35 C7	7.19	0.287	1.943
P18	8.35 C7	8.35 C9	7.25	0.300	1.537
P19	8.35 C8	6.88 C6	7.37	0.527	3.636
P21	6.88 C6	8.35 C8	7.31	0.353	2.593

[1] Optical Density was measured at wavelength of 550 nm, [2] Dry Cell Weight (g/L).

Correlating the data from the experiments performed, we noticed that using a mixture of C8 and C9, a lower amount of dry biomass (g/L) was obtained, compared to the fermentation in which C8 was used as single precursor. When we used a mixture of C8-C6, we noticed that the amount of biomass was higher in the cases that C8 was the first fatty acid added in the bioprocess medium. In fact this combination of precursors (C8-C6), with C8 as first source of carbon added, was the best of all (3.636 g DCW/L).

In the Table 2 are presented the results obtained for polymer composition and purity expressed as g/100 g of analyzed product as determined by GC-FID. After processing the biomass and obtaining PHA, it can be seen that when higher amounts of biomass were achieved (P19, P5, P21), the results were reflected in the amount of polymer obtained (expressed as a percentage, relative to the amount of biomass). The amount of biopolymer contained in biomass was in the range of 36–56%.

Table 2. Percentage values obtained for PHAs biosynthesized, after gas chromatographic analysis.

Samples	PHAs (%)	Hydroxyacids					
		C6 (%)	C7 (%)	C8 (%)	C9 (%)	C10 (%)	C11 (%)
P5	51.16	7.32	-	**88.00**	3.29	1.29	-
P7	40.40	1.25	21.58	13.23	**59.63**	0.75	0.47
P13	47.66	2.91	13.57	33.79	**45.72**	0.93	0.86
P14	35.81	1.33	19.55	14.9	**59.59**	1.44	0.77
P17	43.22	0.11	14.23	0.36	**79.32**	1.44	2.54
P18	48.79	-	**66.18**	0.52	26.77	2.20	0.57
P19	56.29	9.66	-	**79.46**	0.13	7.59	0.96
P21	52.64	8.28	-	**82.65**	-	5.53	1.08

In bioprocesses in which mixed additions C8-C9 were made, the percentage of C9 hydroxy acids was higher (45.72 and 59.59 respectively) than that of C8 hydroxy acids (33.79 and 14.9, respectively); in C7-C9 additions, C7 or C9 hydroxy acids prevailed depending on the precursor initially added; and in the C6-C8 combinations C8 hydroxyacids prevailed, according to the results obtained following gas chromatographic analyzes (Table 2). The analytical results revealed that the highest values obtained for the component hydroxy acids were: 66.18 for C7, between 79.46–88% for C8, and from 45.72 to 79.32% for C9. The highest degree of conversion was achieved by octanoic acid (79.46–88%).

The results obtained for the amount of biomass and, after its processing, for the amount of biopolymer, reveal that the best influence on PHA biosynthesis was achieved by octanoic acid, alone or in combination with hexanoic acid (added to the bioprocess medium after 24 h) as can be seen in Figure 1.

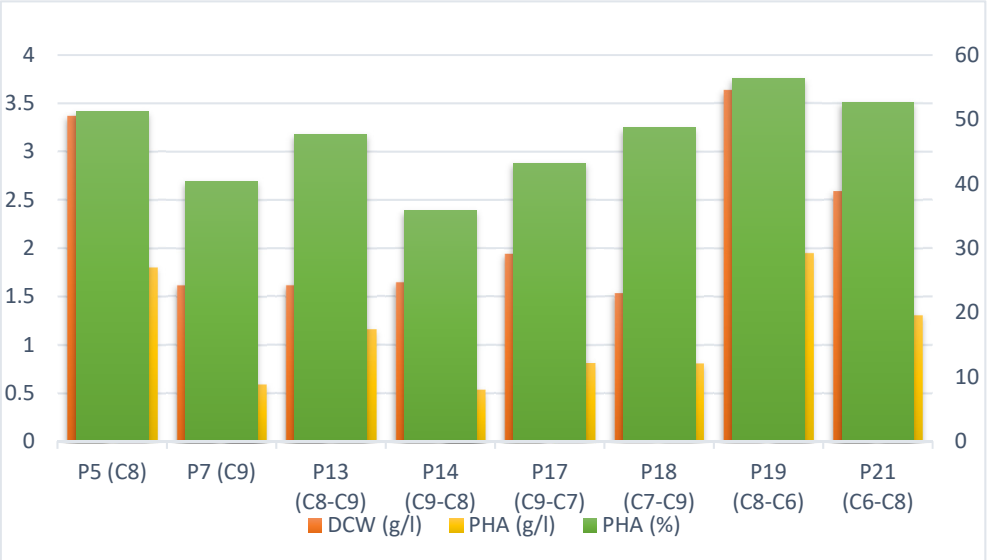

Figure 1. Comparative results on the amount of polymer obtained relative to dry biomass.

4. Conclusions

The highest degree of conversion of fatty acids into biopolymer was achieved for octanoic acid, results also revealed in the amount of biomass obtained.

Nonanoic acid is probably more difficult to metabolize by the microorganism than octanoic acid because, for the same amount added to the bioprocess medium, the biomass resulting from the media containing nonanoic acid was less. This fact is confirmed if we evaluate, comparatively, the supplementation made in the batches with both fatty acids: the amount of PHA obtained was higher when the initial supplementation was made with octanoic acid. The fact that the microorganism (*P. putida*) has a higher affinity for octanoic acid can also be seen if we compare batches 19 (P19) and 21 (P21): the initial supplementation with octanoic acid was beneficial both in terms of the amount of biomass obtained (3.636 g/L), as well as its content in PHA (56.29%).

The results also revealed the performance of the microorganism to produce *mcl*-PHA by converting the monomers tested as precursors to the maximum limit of 16.70 g/L. Thus, the polymers have contained 66.18%—C7, from 79.46% to 88%—C8, from 45.72% to 79.32%—C9.

Following the obtained results, an in-depth study of these biopolymers can be continued for their use as a material in the electrospinning method to obtain fibres and scaffolds for tissue engineering applications.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: This work was supported by the Ministry of Education and Scientific Research, the Grant number: PN-III-40PCCDI/2018 – NAPOLI19 and has been funded by the Operational Programme Human Capital of the Ministry of European Funds through the Financial Agreement 51668/09.07.2019, SMIS code 124705.

References

1. Bhushaniab, J.A.; Anandharamakrishnan, C. Electrospinning and electrospraying techniques: Potential food based applications. *Trends Food Sci. Technol.* **2014**, *38*, 21–23.
2. Lupescu, I.; Eremia, M.C.; Savoiu, G.V.; Spiridon, M.; Panaitescu, D.; Nicolae, C.; Vladu, M.G.; Stefaniu, A. Comparative studies on isolation of medium-chain-length Polyhydroxyxyalkanoates produced by *Pseudomonas* spp. strains. *Rev. Chim.* **2016**, *67*, 1957–1962.
3. Vladu, M.G.; Petrescu, M.M.; Stefaniu, A.; Savoiu, G.; Maria, S.; Eremia, M.C.; Lupescu, I. Studies on polyhydroxyalkanoates biosynthesis by some *Pseudomonas* spp. strains. *Rom. Biotechnol. Lett.* **2019**, *24*, 388–394. [CrossRef]
4. Muangwong, A.; Boontip, T.; Pachimsawat, J.; Napathorn, S.C. Medium chain length polyhydroxyalkanoates consisting primarily of unsaturated 3-hydroxy-5-*cis*-dodecanoate synthesized by newly isolated bacteria using crude glycerol. *Microb. Cell Factories* **2016**, *15*, 55. [CrossRef] [PubMed]
5. Cirstea, D.M.; Stefanescu, M.; Pahonţu, J.M.; Cornea, C.P. Use of some carbon sources by Pseudomonas strains for synthesizing polyhydroxyalkanoates and/or rhamnolipids. *Rom. Biotechnol. Lett.* **2014**, *19*, 9400–9408.
6. Kabilan, S.; Ayyasamy, M.; Jayavel, S.; Paramasamy, G. Pseudomonas sp. as a source of medium chain length polyhydroxyalkanoates for controlled drug delivery: Perspective. *Int. J. Microbiol.* **2012**, *2012*, 317828. [CrossRef] [PubMed]
7. Koller, M. Poly(hydroxyalkanoates) for Food Packaging: Application and Attempts towards Implementation. *Appl. Food Biotechnol.* **2014**, *1*, 3–15.
8. Ali, I.; Jamil, N. Polyhydroxyalkanoates: Current applications in the medical field. *Front. Biol.* **2016**, *11*, 19–27. [CrossRef]
9. Volova, T.; Goncharov, D.; Sukovatyi, A.; Shabanov, A.; Nikolaeva, E.; Shishatskay, E. Electrospinning of polyhydroxyalkanoate fibrous scaffolds: Effects on electrospinning parameters on structure and properties. *J. Biomater. Sci. Polym. Ed.* **2013**, *25*, 370–393. [CrossRef] [PubMed]
10. Puppi, D.; Pecorini, G.; Chiellini, F. Biomedical Processing of Polyhydroxyalkanoates. *Bioengineering* **2019**, *6*, 108. [CrossRef] [PubMed]
11. Li, Z.; Lim, J. Biodegradable polyhydroxyalkanoates nanocarriers for drug delivery applications. In *Stimuli Responsive Polymeric Nanocarriers for Drug Delivery Applications Volume 1*; Woodhead Publishing: Sawston, UK; Cambridge, UK, 2018; pp. 607–634.
12. Koller, M.; Hesse, P.; Bona, R.; Kutschera, C.; Atlic, A.; Braunegg, G. Biosynthesis of High Quality Polyhydroxyalkanoate Co- and Terpolyesters for Potential MedicalApplication. *Macromol. Symp.* **2007**, *253*, 33–39. [CrossRef]