International Journal of Biological Macromolecules 174 (2021) 449–456

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Rapid analysis of polyhydroxyalkanoate contents and its monomer compositions by pyrolysis-gas chromatography combined with mass spectrometry (Py-GC/MS)

Tae Uk Khang [a,1], Min-Jae Kim [b,1], Jee In Yoo [c], Yu Jung Sohn [c], Sang Goo Jeon [b], Si Jae Park [c,*], Jeong-Geol Na [a,*]

[a] Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 04107, Republic of Korea
[b] Korea Institute of Energy Research, 152 Gajeong-ro, Yuseong-gu, Daejeon 34129, Republic of Korea
[c] Division of Chemical Engineering and Materials Science, System Health & Engineering Major in Graduate School (BK21 Plus Program), Ewha Womans University, Seoul 03760, Republic of Korea

ARTICLE INFO

Article history:
Received 4 September 2020
Received in revised form 12 January 2021
Accepted 16 January 2021
Available online 22 January 2021

Keywords:
Pyrolysis-GC/MS
PHB
PHBV

ABSTRACT

Here, we report an analysis method for determining PHA (polyhydroxyalkanoates) contents and their monomer composition in microbial cells based on pyrolysis gas chromatography combined with mass spectrometry (Py-GC/MS). Various kinds of microbial cells accumulating different PHA contents and monomer compositions were prepared through the cultivation of *Ralstonia eutropha* and recombinant *Escherichia coli*. Py-GC/MS could analyse these samples in a short time without complicated pretreatment steps. Characteristic peaks such as 2-butenoic acid, 2-pentenoic acid, and hexadecanoic acid regarding PHA compositions and cell components were identified. Considering constituents of cells and ratios of peak areas of dehydrated monomers to hexadecanoic acid, a simple equation for estimation of PHA contents in microbial cells was derived. Also, monomer compositions of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) in *R. eutropha* could be successfully determined based on peak area of 2-butenoic acid and 2-pentenoic acid of Py-GC/MS, which are the corresponding species of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) in PHBV. Correlation of results between GC-FID and Py-GC/MS could be fitted very well. This method shows similar results for the samples obtained from same experimental conditions, allowing rapid and reliable analysis. Py-GC/MS can be a promising tool to rapidly screen PHA-positive strains based on polymer contents along with monomer compositions.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

Plastics, often referred to as 'miracle material', are ubiquitous and indispensable in our daily lives and modern industry owing to their much low prices and superior material properties. However, indiscriminate use and improper disposal of plastics have yielded environmental threats such as the forming so-called plastic islands and microplastics [1]. Conventional ways of dealing with plastic wastes include recycling, reuse, incineration, and landfill; however, each has limitations in terms of economics and environmental remediation. Recently, biological processes for converting plastic waste-derived scraps and monomers into value-added products —defined as 'upcycling' technology— have been developed to meet both economic and environmental feasibility [2–4]. Nonetheless, it is hard to cope with the enormous amount of plastic wastes being continually generated, relying on the upcycling process alone.

Also, the mass production of plastics is not free from resource depletion and climate change due to its heavy reliance on petroleum-based industry. Therefore, developing a process for the sustainable production of biodegradable plastics would be an assuring solution to the current plastic crisis. In these regards, the biorefinery process has been highlighted more than ever as a promising option to reduce the environmental impact of petroleum-based processes and plastic wastes [5–7]. Since microorganism, a biocatalyst is an integral part of biorefinery, and as powered by recent advances in systems metabolic engineering, microbial cell factories have been extensively developed to produce biodegradable plastics [8].

Especially, polyhydroxyalkanoates (PHAs) have long been examined and regained much attention as they are biomass-derived biodegradable polyesters that can by synthesized by several natural producers such as *Ralstonia eutropha*, *Bacillus* sp., and *Pseudomonas* sp. and engineered bacteria such as recombinant *Escherichia coli* [8–13]. As worldwide, there are more than 20 companies involved in the production of PHAs, and over 15 kinds of PHAs are available on the market.

* Corresponding authors.
E-mail addresses: minjaek@korea.ac.kr (M.-J. Kim), sgjeon@kier.re.kr (S.G. Jeon), parksj93@ewha.ac.kr (S.J. Park), narosu@sogang.ac.kr (J.-G. Na).
[1] These authors are equally contributed to this work.

https://doi.org/10.1016/j.ijbiomac.2021.01.108
0141-8130/© 2021 Elsevier B.V. All rights reserved.