Serbian Biochemical Society

Proceedings

Editor: Ivan Spasojević
Technical support: Jovana Trbojević-Ivić, Milena Dimitrijević, Tijana Ćulafić
Cover design: Zoran Beloševac
Publisher: Faculty of Chemistry, Serbian Biochemical Society
Printed by: Colorgrafx, Belgrade
No of printed copies: 130

Serbian Biochemical Society
Twelfth Conference

International scientific meeting

September 21-23, 2023, Belgrade, Serbia

"***Biochemistry in Biotechnology"***

Evaluation of an in-house developed colorimetric and other assays for PET-degrading activity

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Plastic materials have become indispensable in the modern world, with their extensive use resulting in their environmental accumulation. A promising solution for overcoming this ecological threat may be found in recombinantly produced plastic-degrading enzymes. Due to the complexity of the heterogeneous catalysis occuring during enzymatic PET hydrolysis, quantifying and comparing activities of such enzymes is rendered difficult. Here, we have assessed various assays documented in existing literature, employing different preparations of the purified recombinant *Ideonella sakaiensis* PETase mutant W159H/S238F (expressed from commercial plasmid Addgene #112203). The investigated methods were as follows: p-nitrophenyl acetate (pNA) hydrolysis assay, bis-(2-hydrohxyethyl)-terephthalate (BHET) agar and PET agar diffusion assays, and UV absorbance monitoring after PET particle and PET bottle cut-out hydrolysis. Additionally, we introduced an indirect colorimetric assay using the indicator pyrocatechol violet (PCV). Our work reveals many advantages and problems for each of the tested methods. The pNA hydrolysis assay is the quickest, but many substances which are usually present in enzyme buffers and solutions tend to hydrolyse this compound (e.g. imidazole). It is also unspecific due to hydrolysis by other esterase enzymes. The BHET diffusion assay offers a great tool for activity comparison and estimation, with greater enzyme specificity. However, it is slow and accurate activity quantification is difficult. PET hydrolysis was conducted on in-house prepared PET particles with UV spectrophotometric measurement or by a diffusion assay. Due to the measuring wavelength (240 nm), the importance of proper blanking is critical, but accurate results can still be obtained. The sensitivity of the diffusion assay is much lower in comparison to the similar BHET assay. We also report on a modification of the phenol red indirect colorimetric assay using PCV as the indicator and PET particles as the substrate, which has not been previously described in existing literature.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation Contracts No: 451-03-47/2023-01/200168 and 451-03-47/2023-01/200288.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

577.1(048)

SERBIAN Biochemical Society. International scientific meeting (12 ; 2023 ; Beograd)
"Biochemistry in Biotechnology" : [proceedings] / Serbian Biochemical Society, Twelfth Conference, International scientific meeting, September 21-23, 2023, Belgrade, Serbia ; [editor Ivan Spasojević]. - Belgrade : Faculty of Chemistry : Serbian Biochemical Society, 2023 (Belgrade : Colorgrafx). - 156 str. ; 23 cm

Tiraž 130. - Bibliografija uz većinu apstrakata.

ISBN 978-86-7220-140-6 (FOC)

a) Биохемија -- Апстракти

COBISS.SR-ID 124201993
