Enzymatic oxidation of lignocellulosic biomass-derived furans using novel redox biocatalysts

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Lignocellulosic biomass, a readily available and abundant organic material, is an ideal source to produce high-value compounds that can replace petroleum-based products. Among these, 5-hydroxymethylfurfural (HMF), obtained by the catalytic dehydration of biomass sugars [1], is a significant intermediate that can be converted into valuable compounds including 2,5-furandicarboxylic acid (FDCA), a promising precursor for biopolymers [2]. Biocatalysis offers a environmentally friendly and efficient alternative to traditional chemical methods [3-5]. This study explores the biotransformation of HMF towards its oxidized derivatives using novel fungal enzymes from the Auxiliary Activity family AA5 of CAZy database. Using targeted exploration of fungal genomes, two promising enzymes, a glyoxal oxidase (*Gl*GlyOx) and a galactose oxidase (*Fo*GalOx), were identified and expressed heterologously in *Pichia pastoris*. The recombinant proteins were purified and tested for their ability to oxidize model furans (HMF and its derivative compounds). Our results reveal that both enzymes facilitate the production of oxidized monomers, with *Gl*GlyOx showing efficiency in the biotransformation of HMF to 5-hydroxy-2-furancarboxylic acid (HMFCA) and furan-2,5-dicarbaldehyde (DFF) to 5- formylfurancarboxylic acid (FFCA), while *Fo*GalOx was more efficient in oxidizing HMF to DFF and HMFCA to FFCA. The enzymes were also tested for their ability to transform HMF obtained from real biomass hydrolysates from OxiOrganosolv pretreated wheat straw pulps [6] via enzymatic saccharification and isomerization followed by catalytic dehydration in mild conditions. Various acidic catalysts, including homogeneous (heteropolyacids, organic acids) and heterogeneous (zeolites, supported heteropolyacids) systems were evaluated for their efficiency in dehydrating sugars to furans. Results highlight that the type and ratio of Brønsted to Lewis acidity play a key role in determining the reaction pathways for sugars conversion, significantly influencing the product distribution. This work demonstrates the potential of enzymatic biotransformation as a sustainable route for converting lignocellulosic biomass into valuable chemicals for green polymer production and other industrial applications.

Keywords: biocatalysis, oxidoreductases, furan derivatives, HMF, FDCA, lignocellulose valorisation, heterogeneous catalysis, fructose dehydration

References

[1] Dedes, G., Karnaouri, A., Marianou, A. A., Kalogiannis, K. G., Michailof, C. M., Lappas, A. A., Topakas, E. (2021). Biotechnol Biofuels 14, 172.
[2] Xia, H., Xu, S., Hu, H., An, J., Li, C. (2018). RSC Adv. 8(54), 30875-30886.
[3] Yuan, H., Liu, H., Du, J., Liu, K., Wang, T., Liu, L. (2020). Appl Microbiol Biotechnol 104(2), 527–543.
[4] Cleveland ME, Mathieu Y, Ribeaucourt D, Haon M, Mulyk P, Hein JE, Lafond M, Berrin JG, Brumer H. (2021). *Cell Mol Life Sci.* 78(24), 8187–8208.
[5] Alpdağtaş S., Jankowski N., Urlacher V.B., Koschorreck K. (2024). *Sci Rep.* 14, 5932
[6] Kalogiannis, G., Karnaouri, A., Michailof, C., Tzika, A.M., Asimakopoulou, G., Topakas, E., Lappas, A.A. (2020). Bioresour Technol. 313,123599.

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