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

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INTRODUCTION

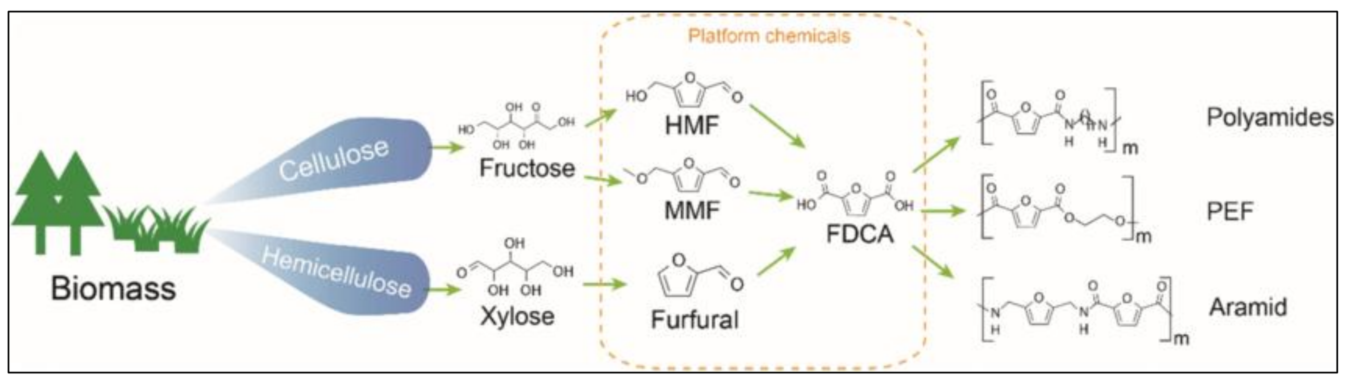
The sustainable production of value-added chemicals from biomass is an increasingly important area of research, particularly for developing renewable building blocks used in bioplastics. Among these, **5-hydroxymethylfurfural (HMF)** has emerged as a key biodegradable and versatile platform molecule as it can be converted into a variety of valuable furan-based compounds through targeted chemical transformations.

A major derivative of HMF is **2,5-furandicarboxylic acid (FDCA)**, which is a critical monomer for producing polyethylene furanoate (PEF) and stands out as a bio-based alternative to terephthalic acid, a conventional component in plastic manufacturing.

The synthesis of FDCA involves a series of **selective oxidation steps**. HMF is first oxidized to intermediates such as 2,5-diformylfuran (DFF) and 5-hydroxymethylfuran-2-carboxylic acid (HMFCA), followed by further oxidation to 5-formylfuran-2-carboxylic acid (FFCA). The final conversion of FFCA yields FDCA, demonstrating a pathway that highlights the feasibility of replacing petroleum-based polymers with renewable, bio-derived alternatives.

SCOPE OF THE STUDY

This research investigates the biotransformation of HMF into its oxidized derivatives using **novel fungal enzymes** from the Auxiliary Activity family AA5 in the CAZy database. Through focused exploration of fungal genomes, two promising enzymes, a **glyoxal oxidase** from *Ganoderma lucidum* (***Gl*GlyOx**) from the AA5_1 family, and one from *Fusarium oxysporum* encoding a **galactose oxidase** (***Fo*GalOx**) from the AA5_2 were identified and successfully expressed in *Pichia pastoris*.



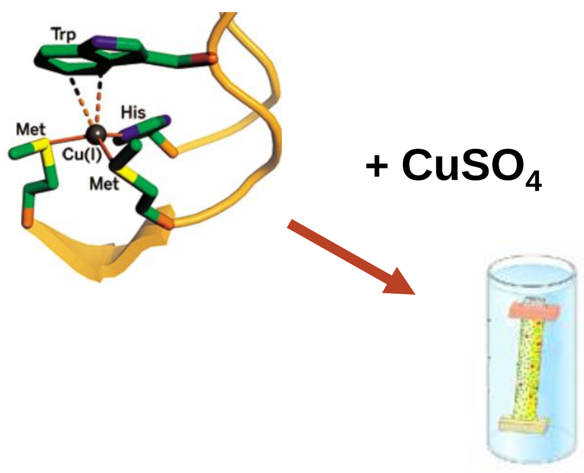
Catalytic conversion of furans derived from plant biomass into various value-added chemicals (Yuan et al., 2019)

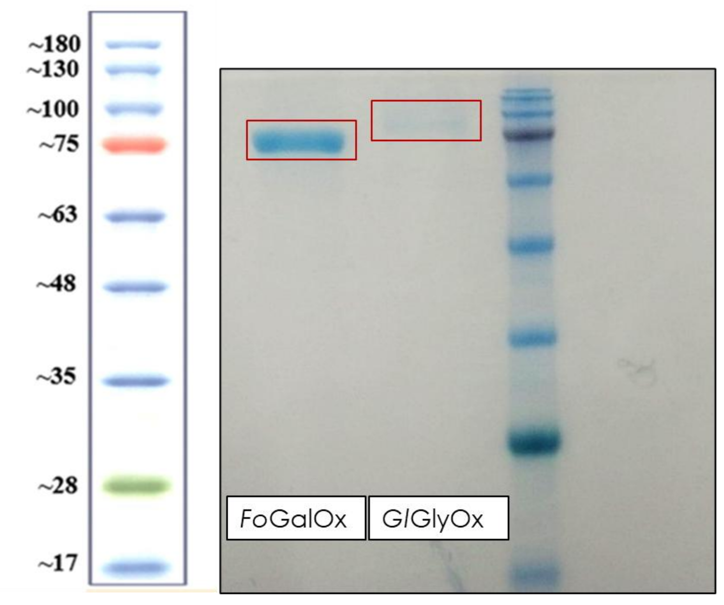
ENZYMES: PRODUCTION, PURIFICATION & ISOLATION

The enzymes were **heterologously expressed** in the methylotrophic yeast *P. pastoris* using the pPICZa plasmid vector. Production was followed in liquid cultures and purification and isolation of the recombinant proteins to their homogeneity was performed with immobilized metal affinity chromatography.

Addition of **$CuSO_4$** followed by dialysis for removal of excess copper ions was necessary for the **activation** of *Gl*GlyOx.

Enzymatic activity was detected using a coupled assay with a commercially available horseradish peroxidase and ABTS. The substrates used for *Gl*GlyOx were glyoxal, methylglyoxal, and glyoxylic acid, while galactose was used as the substrate for *Fo*GalOx.





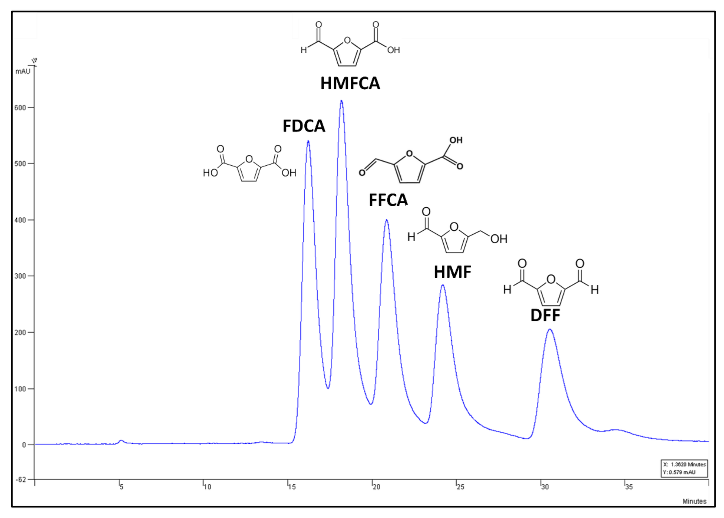
ACTIVITY ON FURANS

The **catalytic activity** of the enzymes on **furan compounds** was assessed using HMF, DFF, HMFCA, and FFCA as substrates. **Catalase** was added to all reactions to relieve the system from H_2O_2 accumulation, and each reaction was performed in duplicate. **Horseradish peroxidase (HRP)** was also included in some of the reactions to assess possible synergistic effects with enzymes and its effect on promoting FDCA production. Reactions were stopped by the addition of a few drops of HCl, followed by product analysis via **high-performance liquid chromatography (HPLC).**

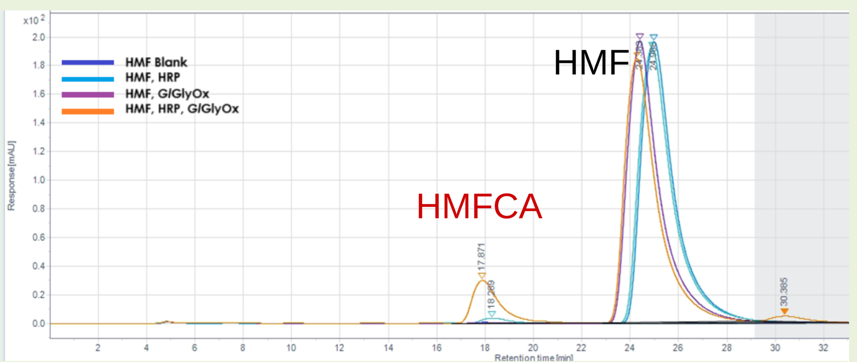
HPLC Conditions

- 0.8 mL/min flow rate
- 60 °C
- λ=264 nm
- Solvent: H_2SO_4 10 mM
- 40 min/ sample

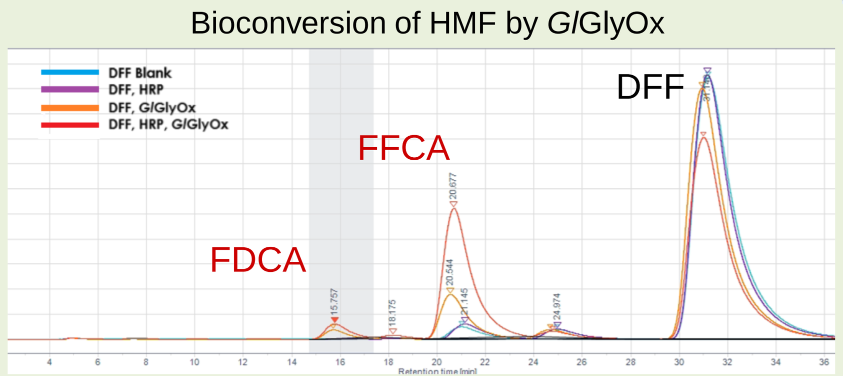
► Elution profile of furan compounds using the Aminex HPX-87H column at 60 °C, with 10 mM H_2SO_4 as the mobile phase and a flow rate of 0.8 mL/min.



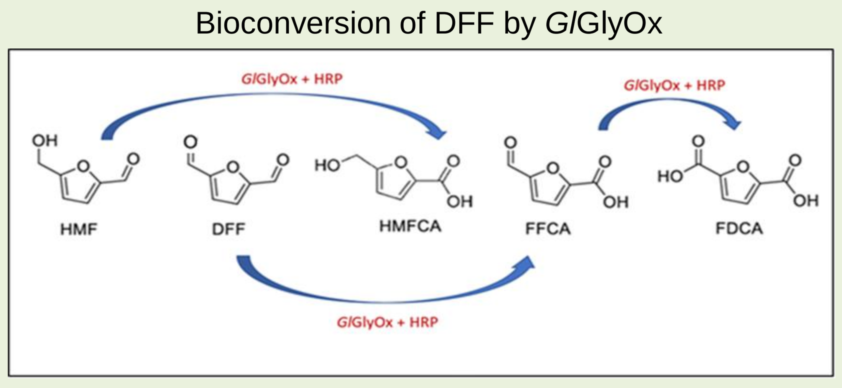
*Gl*GlyOx



Bioconversion of HMF by *Gl*GlyOx

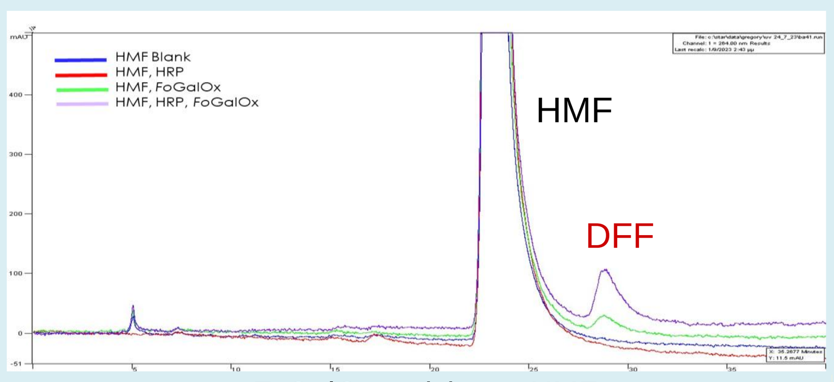


Bioconversion of DFF by *Gl*GlyOx

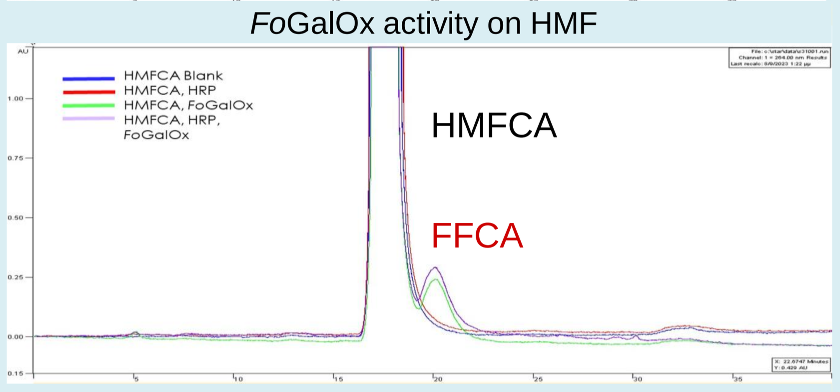


Schematic representation of the activity of ***Gl*GlyOx** enzyme on different furan compounds.

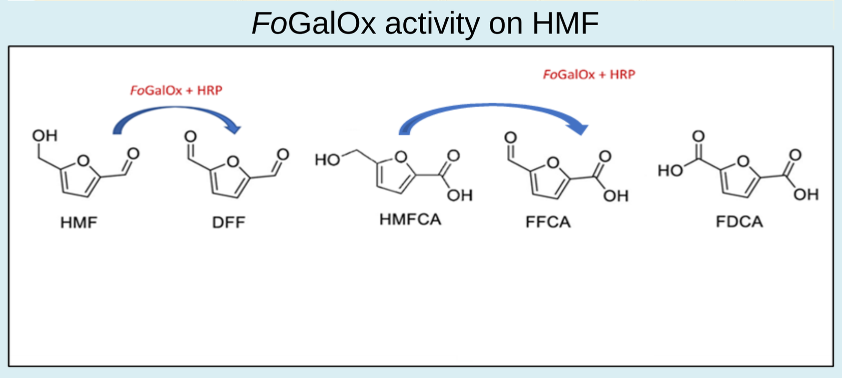
*Fo*GalOx



*Fo*GalOx activity on HMF



*Fo*GalOx activity on HMF



Schematic representation of the activity of ***Fo*GalOx** enzyme on different furan compounds.

CONCLUSIONS

After production, purification, and isolation, the recombinant proteins exhibited **apparent molecular weights** of approximately ~70 kDa for *Gl*GlyOx and ~80 kDa for *Fo*GalOx in contrast to the theoretical molecular weights of 58.69 kDa for *Gl*GlyOx and 72.07 kDa for *Fo*GalOx. The observed increase in the molecular weight of *Gl*GlyOx is likely attributed to **glycosylation** occurring during post-translational modifications in the *P. pastoris* expression system.

Both enzymes demonstrated **catalytic activity** in ABTS-based peroxidase-coupled activity assays.

***Fo*GalOx** exhibited activity towards the substrate HMF, following the oxidative pathway leading to **DFF** formation. Additionally, oxidative activity was observed toward the substrate **HMFCA,** which was further enhanced in the presence of horseradish peroxidase (HRP).

The ***Gl*GlyOx** enzyme required "**activation**" through the addition of copper ions to initiate biotransformation of furan derivatives, as confirmed via HPLC analysis. Upon activation, *Gl*GlyOx displayed catalytic activity toward DFF, resulting in the formation of **FFCA** and catalyzed the conversion of HMF to **HMFCA**. Moreover, *Gl*GlyOx showed catalytic activity to **HMF produced from real lignocellulosic biomass hydrolysates** accompanied by the production of **HMFCA**. In all cases, the presence of HRP significantly enhanced the enzymatic reaction, indicating a synergistic effect between *Gl*GlyOx and HRP.

Given that both enzymes exhibit catalytic activity toward furan-based substrates and their derivatives, they hold potential for individual or **synergistic use** in the biocatalytic production of value-added compounds such as FDCA.

ACKNOWLEDGEMENTS

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