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# DFF: a bio-based cross-linker for the immobilization of enzymes on both conventional and renewable carriers

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#### PURPOSE OF THE ABSTRACT

Glutaraldehyde, a dialdehyde widely used as cross-linker for enzyme immobilization, possesses a widely documented toxicity.[1] Moreover, it has a very complex behavior in water solution: thirteen different forms have been identified, and it is still unclear which is responsible for enzyme immobilization.[2] Finally, it is industrially synthesized starting from fossil sources.[3]

The objective of the present study is to find a renewable and potentially safer substitute for glutaraldehyde for enzyme immobilization. A bio-based dialdehyde has been systematically compared with glutaraldehyde, to determine whether their performances as enzyme cross-linkers and the stability of the final enzymatic preparations are comparable.

The two dialdehydes were compared by immobilizing glucoamylase, a starch-hydrolyzing enzyme, on an amino-functionalized methacrylic carrier. The resulting enzyme preparations were analyzed in activity assays, as well as by a continuous flow experiment, to determine the recovered activity, the stability of the preparation under industrially relevant conditions and thus compare the performance of the two dialdehydes as cross-linkers. The novel cross-linker and glutaraldehyde had very similar performances in terms of recovered activity and stability

of the immobilized enzyme preparations, evidencing the applicability of the new, bio-based molecule for enzyme immobilization in replacement of glutaraldehyde.

The applicability of DFF was verified also studying the immobilization of different hydrolases on a lignocellulosic material. The resulting formulations, besides being fully bio-based, benefit from the lower toxicity of DFF which is less volatile than GA, easy to handle and has the additional advantage of reacting according to clear and simple reaction mechanisms. The latter feature enables its easier dosage as crosslinking agent while minimizing the chemical routes that might cause toxic effects.

## FIGURE 1

## FIGURE 2

#### **KEYWORDS**

immobilization | crosslinker | bio-based

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