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A microreactor with a square shaped reactor chamber was developed with the aim to correlate enzyme positioning with biocatalytic activity. The enzyme position as an important parameter to improve the contribution of the individual enzymes towards the overall reactor efficacy was therefore evaluated experimentally and by computational fluid dynamics (CFD) simulations. Ultimately, such a correlation would lead to faster development through computational pre-screening and optimized experimental design. In this proof-of-concept study, microreactors were prepared in a 2-step curing process of an off-stoichiometric thiol-ene-epoxy (OSTE+) mixture employing both a thiol-ene (TEC) and a thiol-epoxy curing reaction. Subsequent surface functionalization of the remaining thiol groups on the reactor surface through stenciled photoinitiated TEC enabled the preparation of specific surface patterns in the reactor. Patterns were visualized using an allyl-functional disperse red dye, illustrating the successful preparation of a fully reacted surface, a half covered surface and 2 checkerboard patterns. Similarly, allyl glycidyl ether was exploited to functionalize the microreactor surface with epoxide groups, which were used for covalent immobilization of horseradish peroxidase (HRP) in the same patterns. Biocatalytic activity measurements confirmed a clear dependency of the overall reactor performance depending on the spatial distribution of the immobilized enzymes, where specifically the two checkerboard motifs were identified as being particularly effective compared to enzymes covering homogeneously the entire reactor surface. The performance of the same configurations was additionally determined by 3-dimensional CFD simulations. The computational model predicted the same tendencies for the overall reactor performance as obtained from experimental determination. This good agreement between the obtained experimental and computational results confirmed the high potential of CFD models for predicting and optimizing the biocatalytic performance of such a reactor.

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