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Experimental and computational evaluation of area selectively immobilized horseradish peroxidase in a microfluidic device

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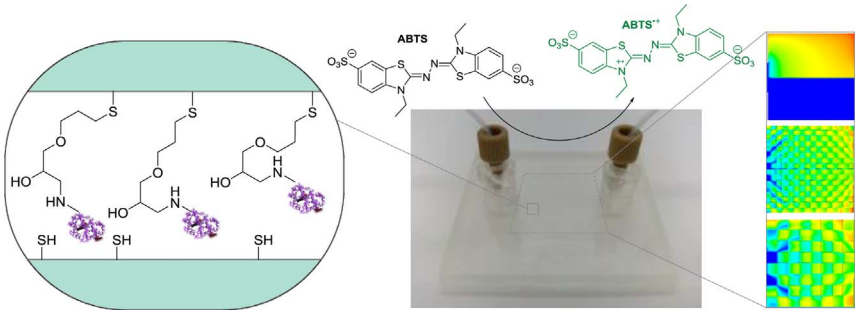
GRAPHICAL ABSTRACT



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ABSTRACT

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

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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.

1. Introduction

The application of enzymes as biocatalysts in industrial processes has attracted increased attention in recent years due to several major advantages of enzymes compared to conventional catalysts. Enzymes originate from renewable resources and take part in green processes with a high catalytic selectivity under mild reaction conditions [1]. These advantageous properties make them attractive for specific applications like synthesis of targeted enantiomerically-pure chiral compounds relevant for pharmaceuticals. However, the disadvantage of enzymes for application at large scale and in continuous processes is their low long-term stability. In order to increase the attractiveness of enzymes for industrial processes, improving their long-term biocatalytic efficiency is a key target. It is well known that an increase in enzyme stability, and consequently biocatalytic productivity, can be achieved through immobilization on solid supports [2–4]. In particular, polymers have shown high potential as enzyme carriers generating biocatalytic systems with an improved stability to external conditions, such as temperature, pH and reaction media [5]. The binding of enzymes on support surfaces can generally be established by adsorption, ionic or covalent bond formation. However, the positive effect in terms of stability is frequently offset by a loss in biocatalytic activity. One explanation is the decreased accessibility to the enzyme's active site by the substrate due to conformational restrictions by attaching the protein to the support material. In order to overcome this, the preservation of the natural environment for a specific enzyme has been identified to be of high importance [6]. This can be realized by chemical modification of the surface, such as by increasing the hydrophilicity of the surface. Functionalization with poly(ethylene glycol) (PEG) for instance shows an improvement in enzymatic activity [7]. Developments in reactor design and reaction conditions play an additional role in order to optimize enzyme efficiency and consequently entire biocatalytic processes. We recently reported that biocatalytic reactors with immobilized enzymes can exhibit differences in their overall productivity, depending on the local position of the enzymes within the flow field of the reactor [8]. Changing the surface pattern of a given amount of enzyme resulted in a different productivity. It was shown that optimization of the spatial distribution of the immobilized enzymes could thus lead to an improved reactor efficiency.

In order to test the influence of such modifications in continuous flow systems, microfluidic devices have been proven to be suitable due to their small size, low consumption of reagents and limited reaction volumes as well as high throughput combined with a high degree of reproducibility [9,10]. Additionally, good heat and mass transfer in combination with laminar flow provide good control over reaction conditions [11]. Laminar flow restricts mixing to occur exclusively by diffusion, unless this is circumvented by specific microchannel configurations. Heterogeneous reactions take place solely on the surface of the catalyst or on the surface that the catalyst is bound to. In these systems, diffusion shows a great impact on the overall reaction, which is influenced by many different parameters, such as flow rate, concentration, dimensions of the reactor geometry and reaction rate [12]. Microfluidic systems are traditionally fabricated in glass or polymeric materials such as poly(methyl methacrylate) (PMMA) or poly(dimethylsiloxane) (PDMS) [13–15]. Especially PDMS has been used extensively for microfluidics due to the ease of fabrication and relatively low cost [16]. Mold casting enables the preparation of different reactor designs and has been applied for the development of biocatalytic microreactors. Hence, enzymes were immobilized on polymer microbeads [17,18] or onto the reactor walls [19]. Alternatively, the formation of a surface patterned phospholipid bi-layer or an adsorbed protein layer [20] enabled the attachment of enzymes on the PDMS surfaces.

Direct protein and enzyme immobilization onto polymer surfaces in microfluidic devices in off-stoichiometric thiol-ene (OSTE) material is an interesting alternative to the currently used PDMS systems. OSTE thermosets have recently evolved into a very versatile platform, which have been successfully applied for fabrication of microfluidic devices [21]. Generally, OSTE systems consist of a multifunctional thiol and a multifunctional alkene (ene) reacting through thiol-ene chemistry (TEC) either under photochemical or thermal conditions [22]. OSTE networks are compatible with many organic solvents, are thermally stable and can be directly surface functionalized. Material properties can be controlled by selecting from a large variety of reagents, which makes this system very versatile. The OSTE approach has recently been updated in order to improve mechanical properties as well as surface bonding, which is essential for the fabrication of microfluidics. In this OSTE+ system, Bisphenol A diglycidyl ether (BADC) undergoes a thiol-epoxy reaction in an additional, orthogonal curing step. This enables covalent sealing between interfaces of the precured material in order to prevent leaking under operating conditions, which is a well-known challenge in microfluidics [23,24]. Mechanical properties of the thermoset can be controlled by stoichiometric variations between thiol and ene groups. Furthermore, off-stoichiometric ratios result in excess of unreacted functional groups, either thiol or ene [25]. These functional groups are well suited for further surface functionalization via photochemical TEC, which has been applied for tailoring surface properties [26,27] or introducing different functional molecules [28]. Additionally, photochemical TEC on the surface offers a possible pathway for surface modification in various patterns by application of a stencil [29,30].

So far, OSTE(+) thermosets have been rarely used for the immobilization of enzymes. Lafleur and coworkers utilized OSTE monoliths for the immobilization of galactose oxidase and peptide-N-glycosidase F after surface activation by means of L-ascorbic acid linking groups [31].

The performance of biocatalytic reactors can generally be improved by different approaches, such as the optimization of reactor architecture and geometries. Recently, Schäpper et al. developed a theoretical topology optimization of immobilization of yeast cells, which resulted in an 8–10-fold improvement in theoretical production rate [32]. Inspired by this approach and by applying isolated enzymes instead of whole cells, we demonstrated using computational fluid dynamic (CFD) simulations that in a laminar flow field of a square reactor with a distributed flow pattern, the biocatalytic reactor efficacy considerably depends on the positioning of the immobilized enzyme [8]. By varying computationally the topology of immobilized enzymes inside the microreactor, simulated reactor configurations with substantially improved productivities were obtained. To the best of our knowledge, reactor optimization based on the positioning of the immobilized biocatalyst within the reactor, either computationally or experimentally, has not yet been done.

The objective of this proof-of-concept study was the methodological development of a biocatalytic reactor, which enabled experimental and computational investigation of the effect of the local distribution of immobilized enzymes towards the overall reactor efficacy. The correlation between experimental and computational results should demonstrate the potential of CFD simulations to predict the biocatalytic performance of such reactors, and would thus open up for the use of CFD for optimization of the microfluidic reactor configuration. In order to achieve this, an enzymatic microreactor containing immobilized