Karlsruhe Institute of Technology

Institute of Functional Interfaces

Reactor Devices for the Compartmentalized Immobilization of Enzymatic Cascades

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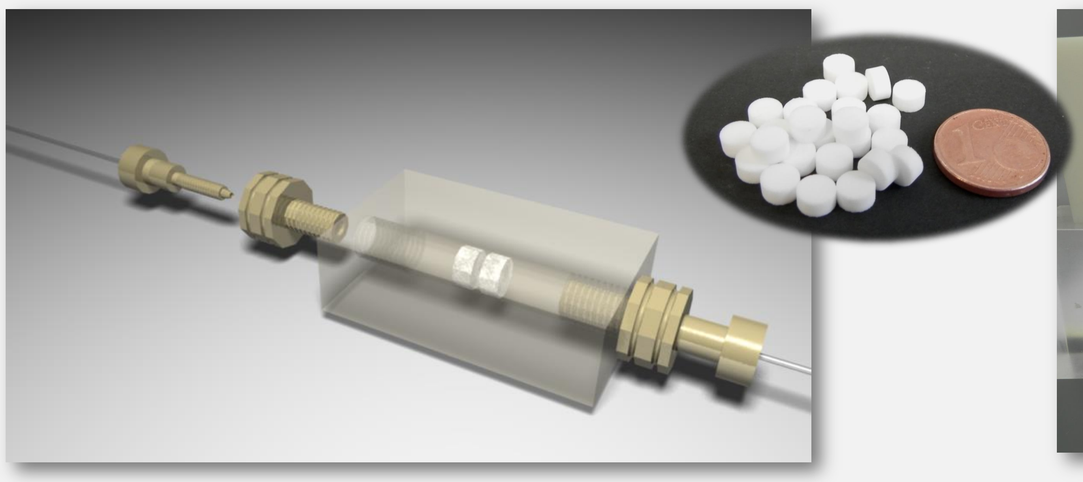
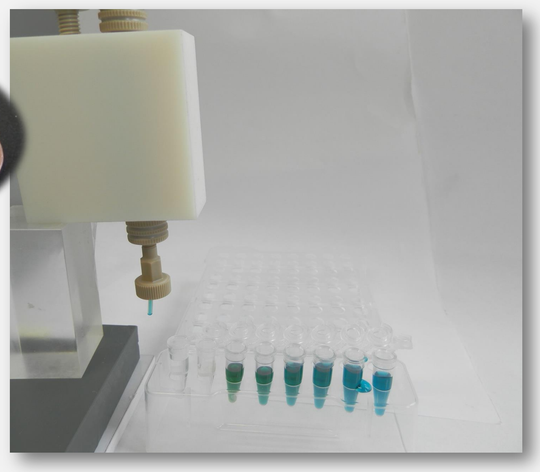
The optimized arrangement of enzymatic cascades in enzyme reactors is an important prerequisite for the synthesis of complex bioproducts within cell free production systems. However, enzymes from different host organisms may differ in their requirements concerning temperature- or pH-optima. To meet these demands the aim of this work is to investigate different approaches for the development of flexible reactor devices for the compartmentalized immobilization of enzymatic cascades.

$$\text{Glucose} + O_2 \xrightarrow[\textit{Aspergillus niger}]{\textbf{Glucose Oxidase}} \text{Gluconolactone} + H_2O_2$$

$$H_2O_2 + TMB_{red} \xrightarrow[\textit{Armoracia rusticana}]{\textbf{Peroxidase}} TMB_{ox} + H_2O$$

Approach I: Compartmentalization by immobilization to functionalized frits

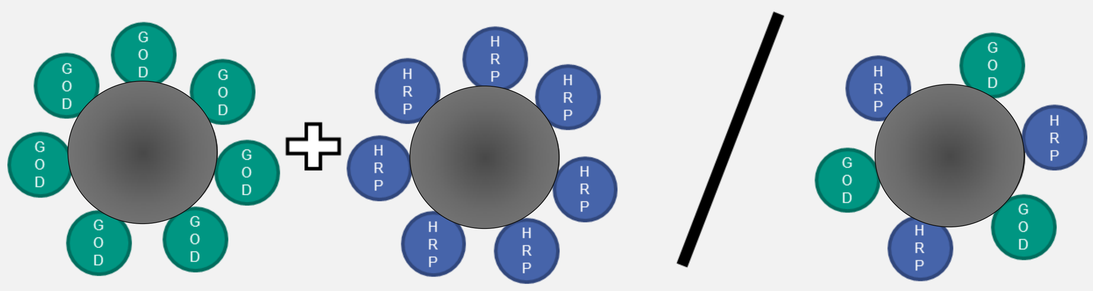




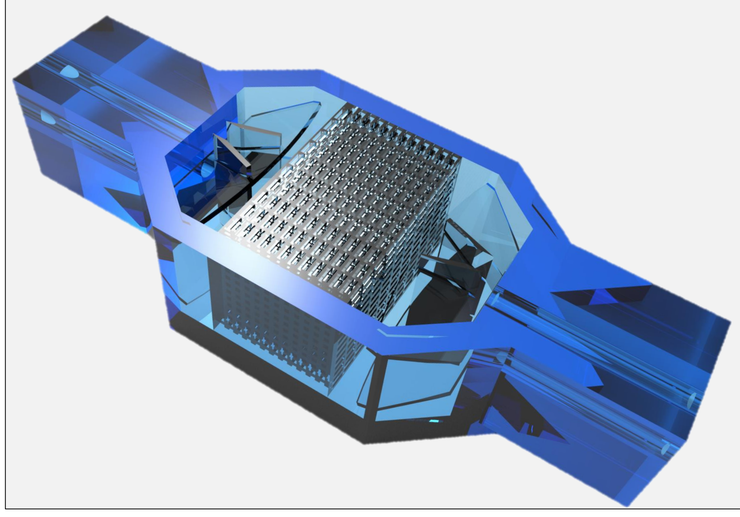
A reactor device for the perfusion of microfilters was designed and manufactured by 3D-printing.
In a first approach, HRP was immobilized to a NH_2-functionalized microfilter by crosslinking. Afterwards the conversion of 3,3‘,5,5‘-tetramethylbenzidine (TMB) could be observed.

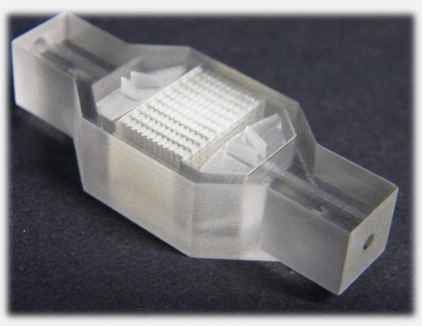
Approach II: Compartmentalization by immobilization to magnetic particles

GOD and HRP were immobilized to magnetic particles. Afterwards particles with co-immobilized enzymes and mixtures of particles with seperately immobilized enzymes were used as glucose sensors to evaluate their performance as cascade.
Both systems detected a broad range of glucose concentrations.
However, the co-immobilized system showed enhanced performance as it was able to detect a broader concentration range.



Approach III: Compartmentalized immobilization within temperature regulated reactor modules





To meet the specific demands of the respective enzymes concerning optimal temperature, disposable reactor modules were designed, that can be individually heated.
The modules were fabricated by 3D-printing, attached to individual Peltier elements and connected in series by a tubing system.
In the next step enzymes will be coupled to the porous gridlike inner structure. By pumping the substrate through the different modules, a conversion of the substrate to the desired product will take place.

Results and outlook

- ✓ Different approaches towards enzymatic cascades immobilized within individual compartments
- ✓ Enzyme immobilisates with high specific surfaces based on porous frits, micro-beads or 3D printed structures
- ➢ Flexible assembling of the compartments into sequential cascades
- ➢ Scalable compartment volumes
- ➢ Optional temperature control, (pH-regulation in preparation) in order to adjust optimum conditions for individual bioreactions within the cascade

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