New Application of Depth Filters for the Immobilization of Lipase

Sarah Schreiber¹, Patrick Jonczyk¹, Axel Thiefs², Ute Schultd³, Lars Dähne³, Thomas Scheper¹, Sascha Beutel¹

¹Institute of Technical Chemistry, Leibniz University Hanover, Callinstr. 5, D-30167 Hannover
²Sartorius Stedim Biotech GmbH, August-Spindler-Str. 11, D-37079 Göttingen
³Surflay Nanotec GmbH, Max-Planck-Str. 3, D-12489 Berlin

Objective

The aim of this study is to immobilize a lipase on modified cellulose-based depth filters. This is done in a circuit with a pump and in order to monitor the protein concentration at 280 nm a flow through quartz cell was used. The chosen biocatalyst Lipase B from Candida antarctica (CalB) from c-LEcta, Germany (Tradename: CalB lyo) is either bound by covalent coupling or physisorption. In both cases, the surfaces of the filter aids, diatomaceous earth and/or perlite, are modified by Layer-by-Layer-Technology (LbL), which is based on the assembly of polyelectrolyte multilayers on charged surfaces. To monitor the successfully conducted immobilization a Bradford protein assay and a para- nitrophenyl acetate (pNpa) assay to determine the enzyme activity were performed.

Layer-by-Layer-Technology

The Layer-by-Layer-Technology uses the electrostatic pull of polyelectrolytes. Therefore charged polyanions can be adsorbed onto a positively charged surface until the surface is reloaded. This procedure is repeated with a polycation which adsorbs onto the prior applied polyanion. This method can be repeated as many times as required and it is also possible to combine different charged materials, such as polyelectrolytes, proteins or nanoparticles. The individual produced layers have a thickness between one and five nanometres.

Results and Conclusion

Various coated filters were tested, differing in the used polyelectrolytes, in charge and in the number of layers (up to three). The used polyelectrolytes are the cationic polyallylamine hydrochloride (PAH) and polyethylenimine (PEI) and the anionic polysulfone sulfate (PSS). The highest immobilization yields (up to 84 %) were reached with negative polyelectrolyte coated surfaces. The highest activity of immobilized enzymes could be obtained using PAH/PSS coating. Furthermore the ion strength plays an important role for a high yield of enzyme binding and as well for the activity. Lower ion strength (20 mM) achieved the best results. For storage and long-term stability tests CalB loaded filters were stored in buffer at room temperature and at 4 °C. The activity was tested after one day, three days, one and two weeks. Bradford assay results showed no protein leaching during the two weeks of storage. Over this time period no significant differences in the activity could be observed. In further experiments the storage will be extended up to eight weeks. Additionally, it was shown that the activity of the CalB decreases by a maximum of 30 % in case of repeated use of the filter.

Acknowledgements

This project is a cooperation between the Institute of Technical Chemistry, Sartorius Stedim Biotech GmbH and Surflay Nanotec GmbH. It has been carried out as an integral part of the Biocatalysis 2021 Cluster, which is financially supported by the BMBF (Bundesministerium für Bildung und Forschung).