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Editorial

Flow Biocatalysis

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The rapid evolution of enzyme technology has enabled new reactions and processes with a level of efficiency which was unimaginable only a few years ago. Protein engineering as well as in silico modelling and de novo enzyme design have dramatically broadened the pool of biocatalysts we can now use for chemistry that goes well beyond the reactions which happen in nature. Enzymes are still perceived, however, as delicate and expensive catalysts and to date only a few biocatalysed reactions have been implemented on an industrial level when compared to the vast majority of "traditional" organic methods.

In the last few years, a new fast-growing field has emerged where enzymatic reactions are shifting from batch conditions to continuous operation mode. Flow chemistry, which is an established methodology in synthetic chemistry, offers a tremendous opportunity to dramatically increase enzymatic performance and bridge the gap from academic explorative research to intensive industrial productivity.

In this special issue, an excellent review from Lindeque and Woodley [1] outlines the importance of reactor designs and selection when it comes to the use of enzymes in continuous settings. While packed-bed reactors are the most commonly used, continuous stirred tank reactors are a valid alternative. The authors point out the different aspects to be considered when choosing a suitable system, parameters such as the enzyme catalytic efficiency, the number of steps in the reactions, the stability, and compatibility of reagents and catalysts could favour one type of reactor over another.

Once the reactor has been identified, the form of the biocatalyst is of course also of exceptional importance. Three research papers are focusing on this challenge. Peschke et al. [2] report on a comparative study of different biocatalytic flow reactor concepts were the (R)-selective alcohol dehydrogenase from *Lactobacillus brevis* was used in a standardised reduction of 5-nitrononane-2,8-dione. The enzyme was self-immobilised on the surface of the reactor via a streptavidin/streptavidin-binding peptide interaction. Three different immobilisation strategies were trialled; as monolayers on the flow channel walls, on magnetic microbeads in a packed-bed format, or as self-assembled all-enzyme hydrogels. The authors thoroughly compare parameters such as space–time yield to enable cross-platform comparisons and concluded that hydrogels offered the best productivity for this system. Molnár et al. [3] describe the efficiency of whole-cell immobilisation for continuous reactions. While certainly the use of cell-free enzymes is more common, whole cells offer the enhanced stability of the biocatalyst within their natural environment. One general concern, however, is the durability of the whole cell system when subjected to continuous flow. Here the authors investigate the immobilisation of *Escherichia coli* cells containing different overexpressed transaminases onto hollow silica microspheres. By comparing the enzymes' performance in the kinetic resolution of four racemic amines in both batch and continuous flow, they showed how the immobilised biocatalysts also maintained excellent activity and selectivity in the flow reactor with sustained productivity for over two days. Similarly, De Vitis et al. [4] exploit whole cell immobilisation for the preparation of enantiomerically enriched 2-hydroxymethylalkanoic acids via the oxidation of achiral 1,3 diols. Here the authors immobilised cells of *Acetobacter aceti* in dry alginate, and again compare the performance in batch and flow modes. As the reaction requires oxygen as the oxidative partner, the implementation of such a reaction in

flow, where the catalyst is compartmentalised in a closed vessel, is challenging. To maximise the oxygenation content in the reaction media flowing through the packed-bed reactor, they implemented a segmented gas–liquid flow regime to maintain high yields and biocatalyst productivity.

Finally, Roura et al. [5] tackle an issue which is often a deal-breaker for enzymatic reactions in flow; the challenge posed by an insoluble starting material. While an increasing number of enzymes has been shown to tolerate significant amounts of co-solvents, in this work, this approach was insufficient in delivering the substrate to the covalently immobilised catalyst. The reaction, the hydrolysis of naproxen ester mediated by a covalently immobilised *Bacillus subtilis* esterase, was dramatically improved by the addition of minimal quantities of surfactants, which enabled higher concentrations of starting material.

In conclusion, this Special Issue showcases different systems and offers an overview of the most common issues when delving into flow biocatalysis.

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