MicroTechEnz

Microreactor technology for continuous enzymatic reactions catalyzed by C-C-bond forming enzymes

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The primary objective of the project 'Microreactor technology for continuous enzymatic reactions catalyzed by C-C-bond forming enzymes' was to take advantage of the green aspect of biocatalysis for the synthesis of chiral 2-hydroxy ketones, and iminocyclitols with high pharmaceutical potential, using enzymatic carboligation and microreactor technology. The project addressed the evaluation of microreactor technology for enzymatic carboligase reactions using thiamine diphosphate (ThDP)-dependent enzymes (TDEs) and D-fructose-6-phosphate aldolase from E. coli (FSA). The further goal of the project was the evaluation of microreactor technology as an appropriate tool for the rapid screening of the enzyme activity and for parameter optimization for the biotechnological production of important compounds.

Setup of the commercial microreactor (Micronit Microfluidics BV) system

Process intensification based on microdevices is a new concept in chemical engineering, which aims at reducing capital and energy costs, as well as environmental impact, by reducing the size of the chemical plant. This project was the first approach using microreactor technology for TDEs and FSA, which both took advantage of this new reactor concept.

'Internal cooperation between the partners during the project was excellent. The consortium meetings were very successful and particularly beneficial for doctoral students. Three doctoral students are now at the end of their doctoral thesis. Three joint publications before the end of the project period are also proof of the excellent cooperation that existed within the multi-disciplinary team. It should be emphasized that self-less knowledge transfer was present at all times, as well as the lending of assistance in the use of equipment. The partners have a good basis for future cooperation, since they have completely gained each other's mutual trust.'

The basic structural unit of a microreactor is a microchannel with typical dimensions between 10 and 500 µm. Due to these small dimensions, microreactors have numerous advantages compared to macroreactor systems. Among these advantages are a decrease in the quantity of chemicals needed and lower levels of energy consumption, which leads to a reduction in the amount of waste, lower reaction times and a high surface to volume ratio, which in turn increases mass and heat transfer. Miniaturization of a process may also increase efficiency, productivity and process safety. Moreover, the small dimensions of a microreactor facilitate reaction screening, which can be carried out without using large amounts of substrates and catalyst.

A further important advantage is that micro-structured devices are operated under continuous flow conditions and therefore can be used as a tool for the optimization of continuous processes. As a result of these advantages, many new microreactor applications are being found, mostly in the fields of medicine and pharmaceuticals.The biocatalysts used in this project were: alcohol dehydrogenases (ADH) derived from Lactococcus brevis and horse liver, NADH oxidase derived from Lactococcus lactis (new enzyme), benzoylformate decarboxylase (BFD) derived from Pseudomonas putida and D-fructose-6-phosphate aldolase (FSA) variants derived from E. coli, all of which were initially prepared in sufficient quantities and kinetically characterized. Benzoylformate decarboxylase (BFD, EC 4.1.1.7) belongs to the group of ThDP-dependent decarboxylases.

The method of BFD-immobilization via a terminal hexa histidine residue (His-tag) on magnetic beads was established. The magnetic beads carry complex Ni2+-ions on the surface that allow coordinative binding of His-tagged enzymes. For the implementation of the reaction with this enzyme in a microreactor, a completely new microfluidic oscillation reactor for enzymes, called µMORE and using magnetic retention and mixing, was successfully designed and put into operation. The µMORE was tested using immobilized BFD, which catalyzes the carboligation of benzaldehyde and acetaldehyde, yielding (S)-2-hydroxy¬propiophenone. Chiral 2-hydroxy ketones are important building blocks or intermediates for the synthesis of several pharmaceutical agents; i.e. bupropion, nitidanin, cytoxazone, anti-depressives and various fungicides. For comparison, the same biotransformation was carried out in a classical continuous laminar microfluidic reactor, with or without the recirculation of BFD and in a continuous enzyme ultrafiltration membrane reactor.

The laminar flow microreactor was shown to be more suitable compared to the ultrafiltration membrane reactor (UFMR) for the continuous process of carboligation reaction of benzaldehyde and acetaldehyde using BFD. If the volume productivities of UFMR and microreactors are compared, it is clear that, regardless of the geometry, laminar flow microreactors have much higher volume productivities. FSA (EC 4.1.2.-) is a novel class I aldolase from E. coli related to the novel group of bacterial transaldolases.

The collection of FSA variants were prepared to find an optimal biocatalyst for the aldol addition of dihydroxyacetone (DHA) to N-Cbz-3-aminopropal, N-Cbz-2-aminoethanal and glycolaldehyde (GO). The product of this aldol addition is the precursor in the chemo-enzymatic synthesis of D-fagomine, a naturally occurring iminosugar with remarkable biological properties. The steady state kinetics characterization for the aldol addition reaction of DHA to N-Cbz-3-aminopropanal catalyzed by the FSA variants A129S and A129S/A165G were performed. Aldol addition of DHA to N-Cbz-3-aminopropanal was carried out in batch, in a continuous enzyme ultrafiltration membrane reactor and in a microreactor. Volume productivity was more than three-fold greater in the microreactor with micromixers than in the batch reactor for the reaction catalyzed by FSA.

Investigation and integration of cascade reactions (i.e. oxidation plus aldol addition with FSA mutants) were performed in a microreactor, as well as in a batch reactor. Oxidation reaction using two methods of coenzyme regeneration (substrate coupled and enzyme coupled) were investigated. For the enzyme coupled approach, a new regenerating enzyme NADH oxidase from Lactococcus lactis was isolated with highly promising results. Four additional methods of oxidation were proposed and tested: i) the system laccase/O2/2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) as mediator; ii) the alcohol oxidase (AO), a flavin-dependent alcohol oxidizing enzyme in the presence of O2; iii) chloroperoxidase (CPO) from Caldaromyces fumago, using H2O2 as an oxidant; iv) a solvent-free oxidation catalyst based on Au/Pd-TiO2 in the presence of O2 or H2O2 were also tested in the oxidation of furfural alcohol.The reduction of (S)-2-hydroxypropiophenone to the respective 1,2-diol was also carried out prior to integration with the carboligation of benzaldehyde and acetaldehyde, as well as the synthesis of C6 substituted carbohydrate analogues by consecutive catalysis of two carboligases: benzaldehydelyase and FSA.