METHODOLOGY TO RAPIDLY ASSESS ENZYME CASCADES IN AID OF METABOLIC ENGINEERING OF HOST CELLS

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Chiral amino alcohols are compounds of pharmaceutical interest as they are building blocks of sphingolipids, antibiotics, and antiviral glycosidase inhibitors. Due to the challenges of chemical synthesis we recently developed two TK-TAm reaction cascades using natural and low cost feedstocks as substrates: a recycling cascade comprising of 2 enzymes and a sequential 3-step enzyme cascade yielding 30% and 1% conversion, respectively. In order to improve the conversion yield and aid the host strain engineering we used a combination of microscale experiments and statistical experimental design. For this we implemented a full factorial design to optimise pH, temperature and buffer type, followed by the implementation of Response Surface Methodology (RSM) for the optimisation of substrates and enzymes concentrations. We achieved 60% conversion for the recycling cascade and 3-fold improvement on the sequential pathway. Based on the results, limiting steps and individual requirements for host cell metabolic integration were identified expanding the understanding of the cascades without implementing extensive optimisation modelling. Therefore, the approach described here is ideal for exploratory work or when the interest is in defining the enzymatic expression levels required for microbial cell factories development.