THE CONSTRUCTION AND VALIDATION OF A PROCESS MODEL FOR A SEVEN ENZYME BIOTRANSFORMATION

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Multi-step enzyme reactions offer considerable cost and productivity benefits. However the optimization of these reactions can be challenging. The construction of process models allows the complexity of these reactions to be understood, and offers a route for their optimization. From a toolbox of characterized enzyme parts, we demonstrate the construction of a process model for a seven enzyme, three step biotransformation using isolated enzymes. Enzymes for the regeneration of the cofactors NAD(P)H and ATP were employed to make this *in vitro* reaction economically viable.

Ensemble modelling was used to propagate experimental error and approximations through the model, allowing confidence intervals to be presented. Furthermore, sensitivity analysis was employed to identify the key parameters contributing most to the model error. We show that the use and validation of process models was instrumental in realizing and removing process bottlenecks, identifying divergent behavior, and for the optimization of the entire reaction using a genetic algorithm. We validated the optimized reaction to demonstrate that complex multi-step reactions of at least seven enzymes can be accurately modelled and optimized.