Development of an *in-vitro* multi-enzyme cascade for the synthesis of uridine diphosphate *N*-acetylgalactosamine using a DoE approach

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Biocatalytic production of active pharmaceutical ingredients, nutritional components and other valuable molecules is becoming increasingly important. In general, either cell-free or whole cell synthesis can be utilized. Both approaches exhibit several advantages and disadvantages (Tao et al., 2011). For instance, whole cell biocatalysts provide inherent co-factor regeneration, whereas in cell-free systems undesired side reactions can be avoided (Straathof et al., 2000). Here, a multi-enzyme cascade producing the nucleotide sugar uridine diphosphate *N*-acetylgalactosamine (UDP-GalNAc) was developed. UDP-GalNAc is an essential building block for synthesis of various glycans and glycoconjugates, such as mucins that fulfill important functions in human health (Figueroa-Lozano et al., 2019; Frenkel et al., 2015). To date UDP-GalNAc is commercially available in mg quantities with prices exceeding 47 €/mg (Sigma, Feb 2024).

The cell-free cascade established consists of six recombinant enzymes overexpressed in *E. coli* (Mahour et al., 2018; 2022). In one-pot batch reactions, the inexpensive substrates uridine and GalNAc are converted to UDP-GalNAc. To additionally reduce costs, ATP is *in situ* regenerated using polyphosphate. Starting at 30 °C, pH 7.5 and 45 mM $MgCl_2$ a conversion yield of approximately 5 % and a final product titer of 1.5 g/L could be obtained within 24 h from initial substrate concentrations of 50 mM uridine and GalNAc, respectively.

Two design of experiments (DoE) sets were employed to increase the product yield. In a first set, temperature (30–40 °C), pH (7–9) and $MgCl_2$ concentration (45–100 mM) and in a second set concentrations of ATP (0.5–20 mM), $PolyP_x$ (20–40 mM) and $MgCl_2$ (80–120 mM) were screened. Optimal conditions within the selected design spaces were identified that resulted in a final UDP-GalNAc titer of 30.4 g/L and yield approaching 100 %. These outcomes demonstrate the effectiveness of a DoE approach and underscore its potential for optimization of multi-enzyme cascades.

References

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