A single-pot enzymatic reaction sequence has been designed for the synthesis of a key intermediate metabolite called D-glyceraldehyde-3-phosphate (D-GAP). The reaction sequence consists of three enzymes and uses D-F16BP as a starting material (Fig.). The rabbit muscle aldolase (RAMA)-catalyzed reaction step is in favor of D-F16BP formation with an equilibrium constant of $10^{-4}$M [1]. Therefore, the single-pot reaction sequence has been designed to shift the equilibrium and regenerate NADH. The capability of the reaction sequence was demonstrated by the enhanced equilibrium conversion from 1.4% to 96%. The reaction system was optimized in detail with regard to activity and stability of the enzymes and stability of the cofactors (NADH and NAD$^+$) and D-GAP. Reaction kinetics models for each of the enzymes were formulated. The effect of co-substances on the activity of the enzymes was evaluated. The results elucidated that D-F16BP and sn-G3P do not influence the activity of FDH and HCOO$^-$ does not affect the activity of RAMA, whereas D-F16BP and HCOO$^-$ suppress the activity of sn-G3PDH. From the kinetics perspective and due to the stability of the enzymes, a continuous operation based on an enzyme membrane reactor was preferred.

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