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Modelling and optimisation of a transketolase-mediated carbon–carbon bond formation reaction

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Abstract

In this paper, we have integrated process characterisation and reaction kinetic data for a transketolase catalysed carbon–carbon bond formation to build a comprehensive reaction model. Based on the synthesis of erythrulose from β-hydroxypyruvate and glycolaldehyde, the model includes component degradation as a function of time and concentration as well as glycolaldehyde toxicity towards the enzyme. Using the ratio of initial substrate concentration as a process variable, simulations and analysis based on this model allowed process options to be evaluated. The model links bioconversion to upstream fermentation for enzyme production and downstream product purification and this could provide guidelines for process development.

Keywords: Mathematical modelling; Biocatalysis; Bioreactors; Biochemical engineering; Process analysis; Transketolase

1. Introduction

In vivo, transketolase (TK) catalyses the reversible transfer of a two-carbon ketol moiety from a ketose to an aldose. Using β-hydroxypyruvate (HPA) as the ketol donor, the enzyme catalyses the transfer of a two-carbon ketol group from HPA to an aldehyde acceptor and requires Mg^{2+} and TPP as cofactor, in which the decarboxylation renders the reaction irreversible (Datta and Racker, 1959), as shown in Fig. 1(a). When glycolaldehyde (GA) is used as the ketol acceptor, the product is erythrulose (ERY) as shown in Fig. 1(b).

Most of the recent work on TK has been carried out using the *Escherichia coli* (Hobbs et al., 1993; French and Ward, 1995; Sprenger et al., 1995; Morris et al., 1996; Lilly et al., 1996) and *Saccharomyces cerevisiae* (Wikner et al., 1994) enzymes, the genes for which have been over-expressed, thereby making available greater quantities of enzyme for biophysical studies and applications in organic synthesis (Turner, 2000).

To date, work on reactor design and operational analyses on the use of TK for carbon–carbon bond formation that have been reported were based on either process characterisation or reaction kinetics. In a previous paper, we applied a structured decision-making procedure (Woodley and Lilly, 1994) to TK-mediated reactions (Woodley et al., 1996) for reactor selection and operational analysis. A number of operational issues such as optimisation of substrate feeding, enzyme immobilisation, and *in situ* product removal (ISPR) were identified. In addition, we (Mitra et al., 1999) have used process information including substrate solubility, component degradation, and reagent toxicity towards the enzyme to identify process constraints and subsequent issues for scale-up. In order to deal with product inhibition, we (Chauhan et al., 1996; Chauhan and Woodley, 1997) described the process using ISPR as a means of improving productivity. As an alternative, Bongs and coworkers (1997) used a membrane reactor to reduce the substrate toxicity towards the enzyme, i.e., control the substrate concentration in the reactor, to achieve higher productivity. Vasic-Racki and coworkers (2003) illustrated the use of a reaction kinetic model to simulate and analyse a cascade membrane reactor. Substrate toxicity towards enzyme was included in their reaction kinetic model.

In many biocatalytic processes, the recovery of product dominates the cost of manufacturing. Reversible product inhibition,

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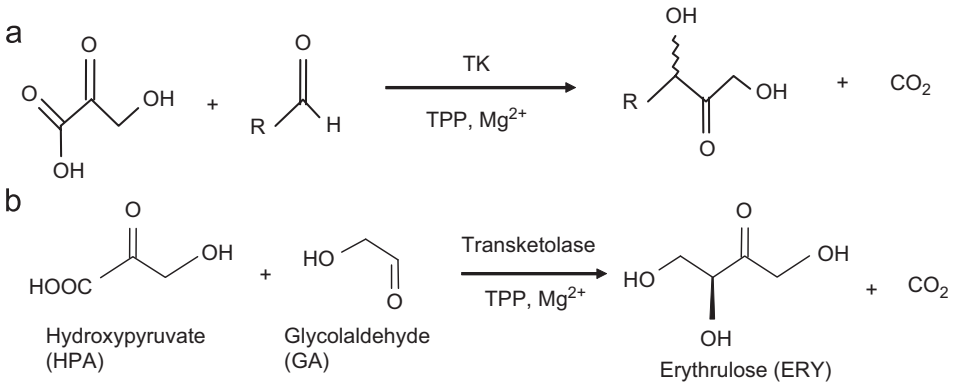

Fig. 1. Reaction scheme of TK mediated carbon–carbon bond formation using β-hydroxypyruvate as ketol donor (a), transketolase-mediated erythrulose (ERY) synthesis via β-hydroxypyruvate (HPA) and glycolaldehyde (GA) reaction (b).

irreversible product toxicity or equilibrium limitations are frequently observed and often limit the achievable product concentration leaving the reactor and providing the feed to the downstream process. Key to overcoming this bottleneck is an understanding of the relationship between reactor conditions and downstream operations and examining such a relationship is best achieved via the use of a process model.

In this paper, we have integrated all the available information –process characterisation and reaction kinetics for a TK catalysed carbon–carbon bond formation to build a comprehensive model. Simulations and analysis based on this model incorporate the impact of initial substrate concentration ratio on reaction rate in a time-dependent manner, which neither the characterisation approaches, nor the reaction kinetics alone are able to. Furthermore, since fermentation and downstream processing were both taken into consideration, such analysis can provide guidelines for process development and potentially cost estimation.

2. TK-mediated reaction modelling

2.1. Modelling the impact of process conditions

2.1.1. Substrates and product pH stability

In a previous study, we investigated the pH stability of the reaction components, HPA, GA and ERY, at different values (in the range of pH 7.0–9.0) and concentrations from 100 to 500 mM at 25 °C (Mitra et al., 1999). The reason for choosing such a pH range is that the optimal pH for TK enzyme activity is pH 7.0–7.5 with > 90% of the maximum activity maintained in the pH range 6.5–8.0 (Mitra et al., 1998). Using these data, empirical models were developed to predict the degradation rates as a function of pH and concentration.

$$\text{For HPA: } D_A = \frac{0.1298\text{pH}^3[\text{A}]}{26549.6 - 2774.6\text{pH} - 0.2409[\text{A}]}. \quad (1a)$$

$$\text{For GA: } D_B = \frac{0.1694\text{pH}^3[\text{B}]}{30026.0 - 3183.1\text{pH} + 0.1511[\text{B}]}. \quad (1b)$$

$$\text{For ERY: } D_Q = \frac{0.1549\text{pH}^3[\text{Q}]}{28873.8 - 2978.1\text{pH} - 0.6426[\text{Q}]}, \quad (1c)$$

where D_i is the degradation rate of component i, and i can be HPA, GA or ERY. [A], [B] and [Q] represent the concentrations of HPA, GA and ERY, respectively, in all equations throughout this paper.

2.1.2. GA toxicity towards enzyme

In previous work, we (Mitra et al., 1998, 1999) and others (Vasic-Racki et al., 2003; Bongs et al., 1997) have reported the phenomena of GA toxicity towards TK. We use inhibition to refer to a reversible loss of activity while toxicity represents an irreversible and time-dependent loss of activity. We used the following equation to empirically model this behaviour by fitting data from Mitra (1997):

$$AR = \frac{\text{Act}}{\text{Act}_0} = \exp\left(-\frac{483.3[\text{B}]}{7.357 \times 10^7 + [\text{B}]}t\right). \quad (2)$$

Its differential form, the same as reported by Vasic-Racki et al. (2003), can be derived as

$$\frac{\text{d}(AR)}{\text{d}t} = -K_{\text{tox}} \exp(-K_{\text{tox}}t) \quad \text{with}$$
$$K_{\text{tox}} = \frac{483.3[\text{B}]}{7.357 \times 10^7 + [\text{B}]}, \quad (2a)$$

where t is time (min), and Act_0 represents the enzyme activity when time $t=0$. The experimental data and model compatibility are illustrated in Fig. 2.

2.2. TK bioconversion reaction kinetic model

The reaction kinetics of the TK-mediated ERY synthesis from HPA and GA follows the ping-pong bi–bi mechanism (Gyamerah and Willetts, 1997) with substrate competitive