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Review

Process technology for multi-enzymatic reaction systems

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ABSTRACT

In recent years, biocatalysis has started to provide an important green tool in synthetic organic chemistry. Currently, the idea of using multi-enzymatic systems for industrial production of chemical compounds becomes increasingly attractive. Recent examples demonstrate the potential of enzymatic synthesis and fermentation as an alternative to chemical-catalysis for the production of pharmaceuticals and fine chemicals. In particular, the use of multiple enzymes is of special interest. However, many challenges remain in the scale-up of a multi-enzymatic system. This review summarizes and discusses the technology options and strategies that are available for the development of multi-enzymatic processes. Some engineering tools, including kinetic models and operating windows, for developing and evaluating such processes are also introduced.

1. Introduction

During the past decade, biocatalysis has become increasingly attractive for the development of more efficient and cleaner chemical synthetic processes (Pollard and Woodley, 2007). Higher selectivity and specificity, as well as the use of mild reaction conditions in general gives an excellent 'green' profile to reactions catalyzed by enzymes. Enzymatic synthesis can either involve a single enzyme catalyzing a given reaction, or can make use of more than one enzyme operating either sequentially or in parallel for the synthesis of often more complex compounds (Fig. 1) (Bruggink et al., 2003). The reactions can be either carried out inside a cell or with isolated enzymes.

In nature, a great number of enzyme cascades can be found in different metabolic pathways inside the cell. The interesting concept of using multi-enzymatic synthesis mimics these chemical processes. In order to systematize these reactions, we propose that the applications of multi-enzymatic systems can be classified into four categories, including cofactor regeneration (parallel reactions), equilibrium shift (sequential reactions), renewable biomass feedstock degradation (mixed reactions) and sugar chemistry (mixed reactions), as listed in Table 1.

The first and most common type of multi-enzymatic application is to those enzymes that require cofactors, such as NAD(P)(H), NTP and others, for effective catalysis. Such cofactors are frequently too expensive for large scale *in-vitro* reactions (Wichmann and Vasic-Racki, 2005). Different regeneration systems have been developed to overcome this difficulty. Among other methods, such as electrochemical, chemical and photochemical regeneration systems, using additional enzymes have attracted a lot of attention in the recent years due to several advantages: (1) mild reaction conditions, (2) effective catalysis, (3) high selectivity, and (4) ease of reaction monitoring (Chenault and Whitesides, 1987). Besides, the reaction equilibrium is often driven towards the desired product and the enantioselectivity of the reaction increases aside from cofactor regeneration (Roessner and Scott, 1996). Nicotinamide cofactors, NAD(P)(H), are the most commonly used oxidoreductase cofactors. NAD(P)H can be regenerated using formate dehydrogenases (EC 1.2.1.2) (Bommarius et al., 1998), glucose dehydrogenases (EC 1.1.1.47) (Patel, 2001; Weckbecker and Hummel, 2005), or alcohol dehydrogenases (EC 1.1.1.1) (Seisser et al., 2007). The reverse reaction, from NAD(P)H to $NAD(P)^+$, can be catalyzed by glutamate dehydrogenases (EC 1.4.1.2) (Römisch et al., 2002; Schultheisz et al., 2008) or NADH oxidases (EC 1.6.X.X) (Riebel et al., 2003). Likewise, nucleoside triphosphates (NTPs) are the cofactors often used in the reactions of glycosynthesis. They can be regenerated from their terminal reaction products, using pyruvate kinases (EC 2.7.1.40) (Koeller and Wong, 2000; Römisch et al., 2002), polyphosphate kinases (EC 2.7.4.1) (Liu et al., 2002), or creatine phosphokinases (EC 2.7.3.2) (Schultheisz et al., 2008) as the catalyst. Nucleoside diphosphates (NDPs) can be regenerated from the monophosphate, using NTP coupled with nucleoside monophosphate kinase (EC 2.7.4.X) (Koeller and Wong, 2000).

The chemical and physical conditions in the enzymatic regeneration systems often require careful selection to maintain the stability of the cofactors. Some cofactors are labile at extreme pH. For example, NAD(P)H can be destroyed at 23 °C in 0.02 N HCl after 1 min, while $NAD(P)^+$ can be decomposed by heating to 100 °C at pH 11 for 15 min (Chenault and Whitesides, 1987). The cost and reusability of the enzymes are also factors of great importance

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