Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Process considerations for the scale-up and implementation of biocatalysis

*Pär Tufvesson, Wenjing Fu, Jacob Skibsted Jensen, John M. Woodley**

Process Engineering and Technology Group, Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Lyngby, Denmark

ABSTRACT

With increasing emphasis on renewable feed-stocks and green chemistry, biocatalytic processes will have an important role in the next generation of industrial processes for chemical production. However, in comparison with conventional industrial chemistry, the use of bioprocesses in general and biocatalysis in particular is a rather young technology. Although significant progress has been made in the implementation of new processes (especially in the pharmaceutical industry) no fixed methods for process design have been established to date. In this paper we present some of the considerations required to scale-up a biocatalytic process and some of the recently developed engineering tools available to assist in this procedure. The tools will have a decisive role in helping to identify bottlenecks in the biocatalytic development process and to justify where to put effort and resources.

Keywords: Biocatalysis; Scale-up; Reactors; Process design

1. Introduction

Today, industrial biotechnology is promoted as a clean, environmentally friendly technology with the potential to transform the chemical industry from petrochemical based oil refineries, using harsh reaction conditions, to the so-called bio-refineries where commodity chemicals can be produced from renewable feed-stocks using mild bioprocesses and thereby contribute to a more sustainable chemical industry (IB-IGT, 2009). Indeed the technology fits very well into the much discussed sustainable chemistry concepts (Jaeger, 2004): the processes are inherently very benign as they are run at moderate temperatures and pressures, using renewable feed-stocks and usually no toxic chemicals in the process.

Industrial biotechnology is already employed in a number of industrial sectors; examples range from animal feed, pulp and paper, leather, detergents, textiles and energy to modifications of starches and fats in the food sector (Kirk et al., 2002), as well as the production of organic and amino acids and vitamins by fermentation (Frazzetto, 2003). However, the focus of the current article is on the use of enzymes (either isolated or immobilised or alternatively contained in 'resting' cells) as catalysts for the synthesis of chemical products—biocatalysis.

Biocatalysts are frequently the preferred choice of catalyst when high selectivity is required. Potentially, introduction of biocatalysis can reduce the total number of processing steps and in particular avoid protection and de-protection steps, leading to a higher atom efficiency (Schmid et al., 2001). Based on published reports reviewing the application of biocatalysis in industry it is clear that the majority of products from industrially implemented biocatalytic processes to date are chiral compounds (Straathof et al., 2002; Schmid et al., 2002; Liese et al., 2006; Pollard and Woodley, 2007). However, biocatalysis is not only interesting for high-value, low-volume, products like chiral pharmaceuticals but also for specialty and effect chemicals, like surfactants, as well as for bulk chemicals and bio-fuels. Indeed the impact of biocatalysis in other industry segments is increasing (for instance recently introduced applications can be found in the manufacture of cosmetic ingredients and polymers).

As the scale of biocatalytic processes increase, more emphasis will be required on the chemical and process engineering considerations, alongside the necessary biotechnological developments. For example, the requirements regarding process intensity and cost reduction are more demanding for high-volume chemicals and bio-fuels, and

* *Corresponding author*. Tel.: +45 4525 2885.
E-mail address: jw@kt.dtu.dk (J.M. Woodley).
Received 10 September 2009; Received in revised form 21 December 2009; Accepted 5 January 2010
0960-3085/$ – see front matter © 2010 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.fbp.2010.01.003

although many different potential processes have been presented in academic literature only a few have made commercial success.

There are several reasons for this:

(1) The biocatalyst is often perceived as too expensive to bring about an economically feasible process.
(2) The development of an optimized biocatalytic process takes a long time and requires many different competencies (as will be shown later).
(3) The probability of success is difficult to estimate.
(4) It is difficult to evaluate the cost of different biocatalytic processes because there is a lack of data on the factors contributing to the total cost.

The development of a biocatalytic procedure at scale is a complex task; it requires broad inter-disciplinary skills and many factors contribute to the final economic competitiveness of the process. Frequently solutions necessitate a compromise between different requirements and therefore good analytical and design tools are required to evaluate the many choices that are presented, to avoid running into dead-ends. In this paper we discuss the considerations required to scale-up and implement biocatalytic processes and describe some of the process engineering tools currently being developed that can be useful in understanding the process to help make rational decisions.

2. Biocatalytic process development

Unlike chemical reaction engineering with as established design paradigm (see Fogler, 2006), to date no procedure for biocatalytic process development has been established. Nevertheless some of the potential steps which should be examined are outlined in the following (Lilly and Woodley, 1996).

2.1. *Reaction characteristics*

A first step in the development of any new reaction scheme is to examine the physical reaction characteristics and determine what constraints these will put on the process. Properties such as reaction thermodynamics and substrate/product solubility and stability under the possible reaction conditions are all important. Estimations of thermodynamic data through computer-based models can assist in finding some of these data.

2.2. *Selection of biocatalyst*

If a reaction has been identified as being a suitable candidate for biocatalysis, the next step is most likely to find an enzyme or enzyme system that is effective for the desired conversion. For some applications, off-the-shelf biocatalysts can be found, but this is most normally not the case. Nature offers a huge diversity of specific enzyme sources and these could be screened for the desired activity. However this could be very time consuming and as many enzyme providers offer screening kits for different types of reactions this would probably be an easier starting point.

When activity for the desired reaction is found, recombinant DNA technology enables the possibility to engineer enzyme functionality (Turner, 2009; Reetz, 2009). The procedure is based on generation of a library of variants that is then screened for the desired properties. The goal is to improve the activity (reaction rate), selectivity and stability of the catalyst. However, a major limitation is that it is generally difficult to simultaneously screen for all of these properties (so-called multi-functional screening) (Burton et al., 2002).

Another critical issue in this step is to effectively manage the almost unlimited number of possible protein variants. Traditional high-throughput screening techniques have limits to the number of variants that can be screened at a reasonable cost. Tools such as ProSAR (Fox and Huisman, 2008) and cluster screening (Vogel, 2007) have been developed to keep the number of screens down.

Recombinant DNA technology tools such as directed evolution have indeed opened up the possible applications and target molecules of biocatalysis and there are several examples where new biocatalytic routes have been established through significant improvement of an existing enzyme via iterative rounds of mutagenesis and screening (Tracewell and Arnold, 2009; Reetz, 2009). To develop a new biocatalyst could be estimated to take about 3–15 months employing a team of skilled scientists (Huisman, 2009). Nevertheless, improvement of the biocatalyst can be essential for many industrial applications. For instance Martin et al. (2007) managed to improve the activity of an aminotransferase by a factor of almost 300, while at the same time improving the stability of the enzyme towards the process conditions, yielding a much more economic process. In another example, Reetz et al. (2006) managed to improve the enantio-selectivity of an epoxide hydrolase from a selectivity factor (E) of around 5–115, by introducing nine mutations to the wild type enzyme.

2.3. *Process development*

Since the chemical industry is under a lot of pressure to reduce processing costs in order to compete in the global market, the performance criteria for a given biocatalytic process are frequently high, and thus the ultimate benchmark for competing technologies will inevitably be cost per kilogram of product. For this reason, both technical and economic indicators should be evaluated when comparing different process options.

The performance of a biocatalytic process is based on a large number of factors. In Fig. 1 a typical biocatalytic process is outlined with some of the most important success factors listed. The efficiency of the fermentation, the form of the biocatalyst, the reaction conditions used, as well as the conditions for downstream product and biocatalyst recovery will strongly influence the performance and the economic sustainability of the process (Burton et al., 2002).

2.4. *Biocatalyst production*

The cost of the whole process is often very dependent on the efficiency of the production of the biocatalyst. A large number of microbial organisms and eukaryotic hosts are available for the production of recombinant protein and different fermentation protocols have been established that allow rapid growth on simple media to produce high cell densities. Combined with the tools to express proteins effectively and in high concentrations, the possibility of obtaining the desired biocatalyst more easily and at a reasonable cost has in recent years increased markedly. Using a fed-batch strategy a cell density of 50–100 g dry cell weight/L with up to 30% by weight being the desired protein can routinely be achieved (Lee, 1996; Vidal