Choice of biocatalyst form for scalable processes

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Abstract
The design of biocatalytic processes for industrial synthetic chemistry is determined in large part by the choice of isolated enzyme or whole-cell catalyst form. In the present paper, the considerations for choice are identified and some important classes of bioconversion are discussed in relation to the choice to be made. Recent developments in cell and protein engineering as well as reactor and process engineering are discussed in addition.

Introduction
Few decisions in the design and development of a new biocatalytic process for industrial implementation are as important as the choice of whether the reaction is to be catalysed (at scale) by an isolated enzyme or by a whole (intact) cell catalyst [1]. Processes implemented before the 1960s were predominantly based on whole-cell catalysis since techniques were not available to efficiently isolate proteins. However, through the 1960s and 1970s, methods for the effective isolation of protein were developed, resulting in the possibility of isolated enzyme operation. Attaching the isolated enzyme to a solid support [2] has allowed reuse of catalyst at the end of each batch, thus reducing the overall cost contribution from the enzyme. The subsequent introduction of rDNA technology (recombinant DNA technology) to enhance protein expression has resulted in still further reductions in catalyst cost [3] and led to implementation of many new biocatalytic processes. Currently, approx. 150 processes are operating industrially with a mix of whole-cell and isolated enzyme catalysts [4–6]. The focus now has shifted to discovery of new enzymes and their implementation in chemical syntheses in industry. For many processes, the development route is complex and in recent years at UCL we have been developing tools to assist in the rapid evaluation of processes [7]. As part of this activity, we have also started to provide guidelines for the evaluation of alternative catalyst forms. In the present paper, I outline some of the issues that need to be considered, techniques to improve particular catalyst forms and tools to enable rapid assessment of different catalyst forms.

Process issues affecting choice of catalyst form
The choice of catalyst form is fundamental to the architecture of the process. In general, isolated enzyme processes require investment upstream of the reactor [8] and whole-cell processes require investment downstream [9]. However, a straightforward upstream/downstream trade-off is often not sufficient to enable a decision to be made. Table 1 lists the key problems when implementing whole-cell and isolated enzyme catalysts.

Table 1: Key problems when implementing whole-cell and isolated enzyme catalysts

Clearly, not all considerations are required for every reaction type and the scientific literature discusses these issues in more detail. Here, I discuss four classes of reaction with particular interest.

Reactions using organic solvent-based media
In the last two decades, work on the use of biocatalysis in the presence of varying amounts and types of organic solvent has been carried out [10], primarily with the goal of enabling higher concentrations of poorly water-soluble reactants to be converted. The more polar solvents damage both cells and enzymes alike [11] although cell softening and potential lysis are a particularly hazard to whole cells. In the case of non-polar solvents at concentrations beyond aqueous saturation, the presence of a second phase may give rise to interfacial damage and emulsification with both catalyst forms (although it has been shown to be partly overcome by immobilization techniques [12,13]).

Oxygen-requiring reactions
Many of the most interesting bioconversions involve the insertion of oxygen into a molecule and therefore require the supply of molecular oxygen [14]. For an isolated enzyme, the requirement is stoichiometric for the conversion, but the presence of oxygen can be harmful to the protein via oxidative damage or interfacial effects [15]. In the case of whole-cell catalysts, the situation is rather different. Here, the primary limitation comes from the need to supply oxygen not just for reaction but also for maintenance (or even growth). This may preclude operation at high catalyst concentrations with the consequent deleterious effects on overall productivity [14]. Where volatile reactants and products are used, this may be of particular concern.

Co-factor-requiring enzymes
Where expensive co-factors (such as NADH or NADPH) are required for enzyme catalysis in stoichiometric amounts,
Developments in cell and protein engineering

The ability to alter the properties of a biocatalyst (via cell or protein engineering) is a powerful characteristic of biological catalysts. Targets to date have focused on alteration of enzymes to improve activity or stability under given conditions [23–25]. Higher activities ultimately mean much reduced fermentation costs. Recently, work has been started on cell engineering, for example altering expression levels of multiple enzyme systems [26]. More work in this activity is required, in particular to devise more stable cells in industrial conditions. While microbial cells may be stable in a given environment in nature, this may not prove the ideal environment for industrial chemistry. Hence, for widespread application, microbial catalysts need to be resistant to industrial environmental features, such as high concentrations of substrate and product, high salt concentrations, organic solvents and extremes of pH. The possibility of altering biocatalysts to have this resistance may come through the use of genetics, screening or selection (for example the use of alternative hosts – Gram-negative organisms have been found to confer greater solvent tolerance than Gram-positive ones [12]). Likewise, it is important that cells can take up substrates effectively. For most non-natural compounds, there is no active transport system and diffusion will determine the rate of access [22] and permeabilization techniques may prove particularly useful [21].

Developments in reactor engineering

The use of immobilization enables isolated enzymes to be effectively recovered from a biocatalytic reaction and recycled. For commercial operation, hundreds of recycles are usually required. Even today there are a relatively limited number of immobilization supports, although some new methods involving crystallization of proteins and aggregates are becoming available. Protein adsorption is of itself a simple operation but the key to multiple recycles is the need to fix the protein firmly to the surface and this requires covalent bond formation. The use of a supported enzyme also gives the possibility of other reactor types such as packed beds and fluidized beds to be used. In contrast, whole-cell catalyst operation is usually carried out in a single batch in an agitated tank, with the catalyst thrown away at the end of the conversion.

Developments in process engineering

Isolated enzyme catalysts have long had the virtue of easy control of kinetics. Addition of known amounts of catalyst to the reactor gives a known rate of reaction. However, for whole cells, catalyst concentration is dependent on that which comes from the fermentation. The advent of high-cell-density fermentation, in particular for *Escherichia coli*, into which many enzymes have been cloned, means that the upstream stages can now be more cost-effective. However, much of the benefit of this is lost if reactions cannot be carried out at the same concentration in an effective way. In order to achieve this, it is preferable if the optimization of the fermentation and bioconversion are independent. Separating the two operations means that media as well as concentration can be changed after fermentation, reducing both bioconversion and downstream costs [27]. A number of industrial processes (some at very large scale) now operate this strategy.

A decision-making tool

As biocatalysis finds wider application, the need to make choices about biocatalyst form will increase. Some of these choices will be straightforward, while others will
require an upstream–downstream trade-off or more complex
decision-making. In an attempt to make such decision-
making more systematic, we have begun the development of
a decision-making tool. The objective behind this is to
to enable decisions to be made as early as possible in process
development such that effort can be focused on a limited
number of key experiments.

Concluding remarks
While in the past the upstream costs were usually dominant
in a biocatalytic process, in recent years the development of
rDNA technology has brought these costs down. For
pharmaceutical products, the molecules have become more
complex and frequently reactant conversion becomes the
dominating issue. While this may take the pressure off catalyst
production and on to downstream processing, it also argues
for implementation of isolated enzyme catalysts. For many
existing pharmaceutical products, this is the case. However,
for the future, more emphasis will probably be placed on
whole-cell catalysis [28–30]. First, pharmaceutical processes
are becoming ever more complex with molecules containing
multiple chiral centres. In these cases, enzyme cascades are
being used increasingly. A recent example uses both whole-
cell and isolated enzymes together in subsequent steps of
a synthesis [31] and increasingly metabolic engineering will
come to bear. Secondly, biocatalysis is now expanding beyond
the pharmaceutical sector where the balance of costs is
different. Use of biocatalysis for the production of bulk
chemicals will place a huge burden on costs and, here too,
processes are only likely to be sustainable via implementation
of whole-cell catalysts. It is impossible to generalize about
what makes the ideal catalyst (and the form of that catalyst) in
a given process [32,33], but while the balance of processes may
move to whole-cell catalysis, tools for effective evaluation in
given case will increasingly be required using methods such as
software tools, miniaturized experimentation [34] and flow
cytometry to assess whole cells [35].

References
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