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A future perspective on the role of industrial biotechnology for chemicals production

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ABSTRACT

The development of recombinant DNA technology, the need for renewable raw materials and a green, sustainable profile for future chemical processes have been major drivers in the implementation of industrial biotechnology. The use of industrial biotechnology for the production of chemicals is well established in the pharmaceutical industry but is moving down the value chain toward bulk chemicals. Chemical engineers will have an essential role in the development of new processes where the need is for new design methods for effective implementation, just as much as new technology. Most interesting is that the design of these processes relies on an integrated approach of biocatalyst and process engineering.

Keywords: Industrial biotechnology; Biocatalysis; Protein engineering; Process design

1. Introduction

This year the European Federation of Chemical Engineering (EFCE) celebrates sixty years of contributions to the chemical industry and the (technical) universities that support that industry. The last sixty years have seen enormous developments in the chemical industry and the discipline of chemical engineering, as reflected by the other papers in this special issue, and Europe has had an important role to play in these developments. By strange coincidence this year also marks the sixtieth anniversary of the discovery of the structure of deoxyribonucleic acid (DNA) (the double-helix) by Watson and Crick in Cambridge (UK). It is this discovery (for which they were jointly awarded the Nobel Prize in 1962, together with Maurice Wilkins) which, more than any other, has led to the development of a new branch of chemical engineering. Today, industrial biotechnology is a major global industry and chemical engineers have not only contributed to this astonishing development but will become increasingly involved in the future too. Interestingly bioprocessing is an area of innovation that leads not only to improved processes for existing products but also a range of entirely new products. Since the discovery of the structure of DNA, it took a further twenty years for molecular biologists to develop the set of tools we today refer to as recombinant DNA technology. Such tools enable access to new expression hosts, overexpression of genes, overproduction of proteins and pathway manipulation, as well as the design of proteins with new properties (Table 1). It is this development which has been such a huge driver for industrial biotechnology enabling new routes to chemical products via cheaper and tailor made biocatalysts (whether in growing cell, resting cell or isolated enzyme format). A second driver is the need to replace fossil resources and over time move to renewable raw materials. An increasing recognition that we have limited resources of fossil-based feed-stocks, metals and other reagents means that a new wave of processes is being developed based on sugar and vegetable oils as well as waste oils and fats. Such feed-stocks are very well suited to bio-based processing methods, since the molecules are already highly functionalized. The third driver is the need for green (clean) processes, where minimum waste is produced as well as efficient use is made of the energy used in producing the product.

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Table 1 – Process implications of rDNA technology.

Tool	Process benefits
Overexpressed protein	Cheaper biocatalyst production
Transfer between hosts organisms	Cheaper biocatalyst production
Alteration of cellular pathways	Designer biocatalysts (reactions)
Insertion of new pathways	Designer biocatalysts (reactions)
Protein engineering	Designer biocatalysts (properties)

All three drivers (i.e. rDNA technology, raw material change and environmental impact) contribute ultimately to the overall economics of the process, and hence product. Obviously it will usually be the most economical solution to a given synthetic problem which will be implemented. Indeed a route with few steps and low environmental impact has the highest chance of success, no matter which technology is employed. In this brief article the motivation for the further development of industrial biotechnology for chemicals production will be discussed together with an outline of the specific role to be played by chemical engineers.

2. Types of bioprocess

There are of course many ways of classifying the range of chemical and biochemical processes of potential use in manufacturing industry today to produce the enormous diversity of products available and useful for society. However one instructive classification is to analyze the nature of the feed-stock, reaction catalyst and product. Fig. 1 illustrates the range of conceptual conventional and new processes possible. Conventional processes use fossil-based feed-stocks and chemical catalysts to produce chemical products. Fig. 1 also shows that chemicals can be made with chemical catalysts but using new routes from renewable feed-stocks. This is an increasingly important area of technology, although beyond the scope of this article. However, with a biological catalyst (either using bio-catalysis or fermentation) the options are increased. For example the feed-stock can be fossil-based but may alternatively be renewable. Likewise the product may be a chemical or alternatively a larger biomolecule (an enzyme, protein, antibody or peptide). These so-called 'bio-products' (including biopharmaceutical, biologic and bio-similar products) have an important market but the scope of this paper will be solely on chemical products. Interestingly, the process to produce some products can be classified in more than one concept. For example biodiesel (the fatty acid alky ester resulting from the trans-esterification of vegetable oil) is considered a bio-based chemical. The raw material feed-stock for the manufacture of biodiesel can come from highly renewable resources (such as waste oil or algae), all the way to refined vegetable oils (in competition sometimes with food – renewable but not sustainable). Furthermore the reaction catalyst can be either enzymatic, chemical (alkaline) or a combination. Even the co-substrate alcohol (methanol or ethanol) could in the case of ethanol be from a renewable source (i.e. bio-ethanol via fermentation of sugar or lignocellulose) (Severson et al., 2013). This example illustrates well the complexity of classifying such systems. Nevertheless it is instructive to make some type of classification. In the future it will be increasingly important in order to understand the drivers for changing from conventional systems. Many of these drivers for change will set the agenda for the next generation of manufacturing processes. In this paper the scope is solely about concepts 3 and 4 (Fig. 1).

3. Drivers for change

3.1. *Recombinant DNA technology*

The development of recombinant DNA (rDNA) technology enables several possibilities for the real exploitation of biocatalysts in the full sense of the word. First it has provided

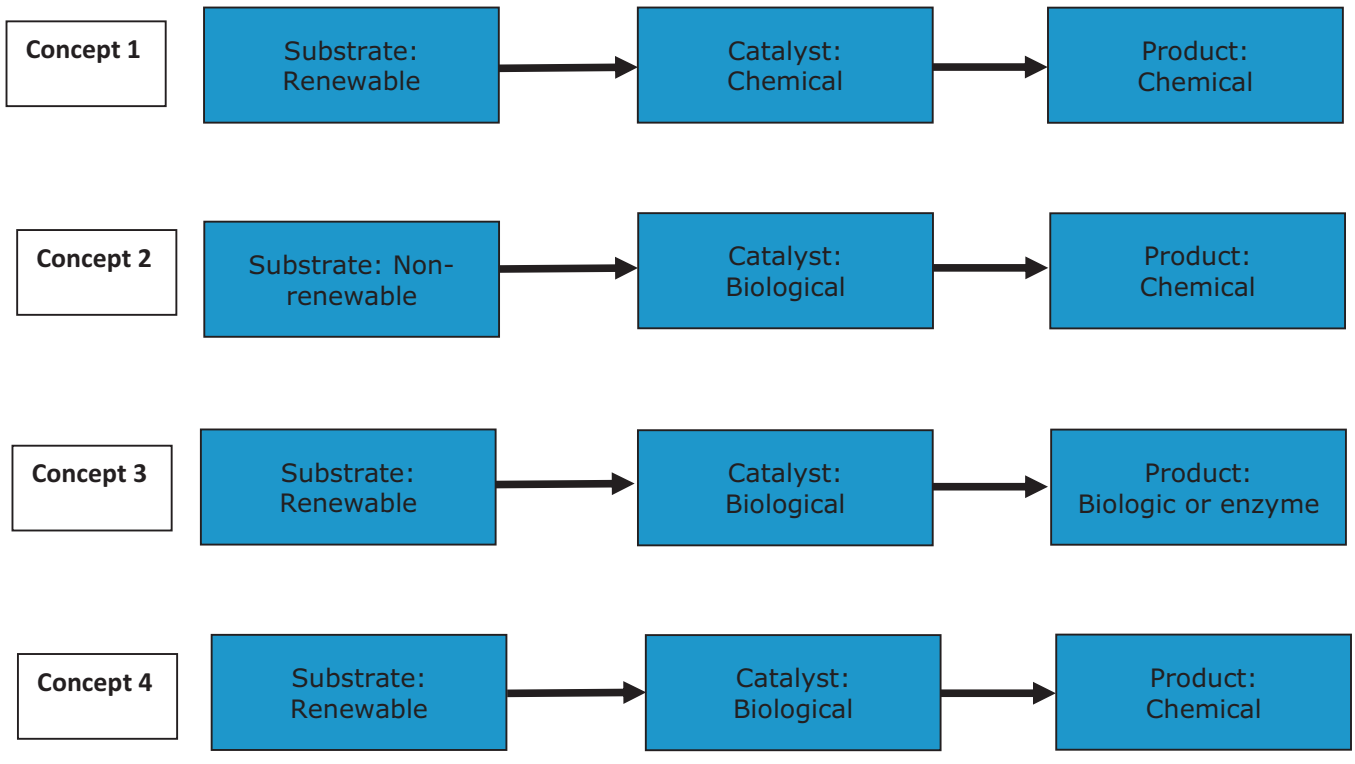

Fig. 1 – Potential classification of bioprocesses.

a cheap way to produce a given biocatalyst. The desired enzyme (or enzymes) can now be overproduced meaning that it represents a much bigger fraction of the available protein in the cell. This not only reduces the required scale of the fermentation (and consequently the feed-stock and energy required, as well as the waste produced) but for isolated enzyme applications also reduces the downstream burden prior to catalysis. Secondly a given gene may be expressed in vivo only in a poor host for production (e.g. the host may be pathogenic or grows only under conditions far from those used for application). Such a situation can be overcome by genetic engineering through codon optimization and subsequent cloning the desired gene into a new more appropriate host organism. Typical hosts in an industrial setting are for example *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* or *Pichia pastoris* since they are fast growing (overcoming the risk of contamination) and the genetics are well understood, although others are used, dependent upon application. In some cases protein secretion is possible (e.g. from *Aspergillus niger*). Each of these developments has helped to revolutionize the biotechnology industry since they enable proteins and biocatalysts to be provided at a reasonable cost. The ability to grow cells to a high concentration (high cell 'density') based on sophisticated fed-batch feeding profiles has also had a major impact. Recombinant DNA technology can also enable an alteration of the properties of the biocatalyst. For cells this can involve alteration of pathways (blocking non-productive routes) and increasing metabolic flux or even creating *de-novo* pathways (Jones Prather and Martin, 2008; Meyer et al., 2007). Today synthetic biology is a hugely exciting area of industrial biotechnology which will develop entirely new routes to chemicals. Whether it is to be carried out inside or outside the cell is still an open question. In some cases compartmentalization is useful and in other cases not. For enzymes, the ability to swap the amino acids either in the active site or even at remote positions of the protein has been found capable of altering and controlling substrate repertoire, enzyme stability, activity (reaction rate) and selectivity. Today, protein engineers routinely use so-called 'directed evolution' as well as rational strategies based on protein structure information to optimize proteins (Strohmeier et al., 2011; Reetz, 2013). For the future this will be applied in processes at full scale. Examples already exist but it is clear this is a very exciting area that will develop enormously in the coming decades. The input of chemical and biochemical engineers is highly important here, because they alone can set the agenda for protein engineers, dependent upon process requirements.

3.2. *Renewable raw materials and reagents*

A major preoccupation of the chemical industry in the last decade has been the development of alternative chemical production processes based on renewable feed-stocks, meaning those not derived from fossil or oil-based chemicals (Gwehenberger and Narodoslawsky, 2008). Such a shift is driven by increasing oil prices as well as the need for reducing dependence on a single source. Ultimately oil will run out and need replacing, and although there remains time till this takes place planning for the change now is important. The argument has mostly focused on the need for an alternative fuel source, which brings with it the huge challenge of making a product which can only be sold at a very low price. One approach has been the development of a so called 'bio-refinery' that supplements the cost of producing low value biofuel with higher value bio-based chemicals. In recent years the US Department of Energy has even defined a list of the top building block chemicals of importance for a bio-refinery based on renewable carbohydrates (Werpy and Petersen, 2004). For example succinic acid is one such chemical on the verge of commercialization. While it is not clear where such a debate will end it is evident that the use of carbohydrates as a feed-stock implicates fermentation and biocatalysis as likely, even if not the only, methods in producing such chemicals, complemented by chemo-catalysis (Bozell and Petersen, 2010; Dapsens et al., 2012; Thomas et al., 2002; Marr and Liu, 2011; Herrera, 2004).

3.3. *Green processes*

Good chemical engineering has long been recognized as that required to create, develop and design cost-effective processes for chemical manufacture. In the last sixty years the demands on manufacturing have developed such that today a second set of requirements is that processes have also to meet strict safety requirements, both for those involved in the manufacture of such products as well as those that use the products. It has been an important development, but now in the next sixty years a third requirement will be integrated with these – the environmental (and with it the societal) impact of the process. To some extent the integration of this third requirement is already underway but it needs further development. For example, aside from the driver toward renewable feed-stocks and reagents, there is also a trend toward manufacturing processes with minimal waste and which use reagents in a very effective way (Gwehenberger and Narodoslawsky, 2008). Ultimately this will lead to sustainable chemical processes and products. Industrial biotechnology is one tool available to achieve this although it is clearly not the only one. However a necessity in developing sustainable processes in the future will be that appropriate measurements are made of the 'eco-footprint' of the process. Today many metrics are used to assess such processes (Dreyer et al., 2003; Saling et al., 2002; Andraos, 2013), from full life-cycle analysis (LCA) to green chemistry metrics (GCMs) (e.g. PMI; Kjell et al., 2013) or E-factor (kg waste/kg product) (Sheldon, 2007). While a range of metrics, models and software tools are available today (Shonnard et al., 2003; Jiménez-González et al., 2013), they need to be developed further (and potentially standardized) to match every stage of development and be used to guide development and process improvement. For example the tools to be used at an early conceptual stage when limited data are available are clearly different to those to be used at a late stage of development, when detailed design is carried out. It is frequently argued that bioprocesses are cleaner than their chemical counterparts, but aside from the few isolated examples where comparisons have been undertaken (Vink et al., 2010; Henderson et al., 2008), this argument must not be generalized. Many bioprocesses are highly selective and operate with low (Henderson et al., 2008) energy requirements (during operation) but are heavy on water usage and downstream processing. For the future making such comparisons will be important to build up a significant database of cases, such that expectations can be met (Jiménez-González et al., 2011; Adlercreutz et al., 2010). Likewise integration into existing chemical factories may come at an environmental price due to the differing conditions required for chemical and biological reactions (Wenda et al., 2011).

Table 2 – Process features of biocatalytic processes.

Advantages
- Selective chemistry
- Mild condition reaction conditions
- Water based reactions, minimizing organic solvent use
- Integration with other bioprocessing steps due to compatible reaction conditions

Disadvantages
- Catalyst and process needs to be engineered to match industrial requirements
- Catalyst need to be engineered to enable conversion of non-natural substrates
- Liquid based reactions
- Integration with chemical steps due to incompatability of reaction conditions

Table 3 – Examples of potential challenges in the industrial application of biocatalytic processes.

- Availability of some biocatalysts at industrially relevant scale.
- Selection of biocatalyst format (e.g. isolated enzyme, immobilized enzyme, whole-cell).
- Time taken to 'evolve' or screen the biocatalyst to operate at the required rate with non-natural substrates at the appropriate substrate and product concentrations.
- Time taken to integrate process engineering solutions with the biocatalyst properties to achieve 'optimal' process configuration and operating strategy.

4. Current bioprocess technology

Although many of the principles of biochemical engineering are identical to those of chemical engineering, involving transport phenomena, thermodynamics, reaction rate laws and separation principles, combined with mass and energy balances, there are several features of bioprocesses which are special (Yuryev and Liese, 2010). It is these special features of industrial biotechnology which perhaps more than anything else distinguish biochemical engineering as a separate discipline.

- Unlike many chemical reactions (which often take place in the gas phase) many of the reactions which constitute the synthetic stage of bioprocesses take place in liquid phase, frequently water. This means that transport phenomena are different and it also has implications for the recovery of products from such media. This is particularly critical given that in nature bioprocesses work in dilute conditions, meaning that integration into existing processes is frequently difficult (or requires large investments). In all cases for industrial application it is necessary to engineer either the biocatalyst to cope with higher concentrations or the process to overcome low concentrations, or a combination of both. Given the need for high product concentration to keep the downstream and waste costs manageable, it will also be necessary to address higher substrate concentrations as well.
- Any heat produced in an exothermic reaction (e.g. in an aerobic fermentation) will be at ambient temperature (or maybe a little above). This means heat integration with the downstream process is difficult, since this is not useful heat.
- Frequently (and especially in fermentation) the product is not the major component to enter the downstream process. This situation places particular emphasis on the selection of effective unit operations in the downstream process.
- The interaction between unit operations is particularly strong, meaning that changes upstream (in the fermentation or biocatalysis stage) can have implications further downstream, where the product is recovered, purified and polished. Indeed often there are changes downstream simply because the output from the fermentation is not consistent.

Table 2 lists these key features and the implications for engineering the process and the biocatalyst. It is clear that chemical and biochemical engineers have an important role to play in addressing these process problems. This also places particular emphasis on the development processes itself, where chemical engineers need to work hand-in-hand with biochemists and microbiologists at an early stage of development.

4.1. *Industrial fermentation processes*

Industrial fermentation is a multi-step processes where cultivation is increased in scale in a staged approach. Today most industrial fermentation products are produced at the largest scale via fed-batch strategies. Limiting a carbon source in this way by feeding is used to slow down the fermentation in the latter stages, with the advantage that oxygen supply and heat removal can be managed. Nevertheless for aerobic fermentations the size of an individual unit is limited to around 200 m^3, making it hard to gain the benefits of economies of scale seen in conventional petrochemical processes. For anaerobic fermentation the yield of product is often, although not always, lower (since more of the carbon goes into the cells and other products). Nevertheless, final the scale can be as high as 500–1000 m^3. A plethora of chemical products (Miller and Nagarajan, 2000; Whited et al., 2010; Weusthuis et al., 2011) are produced by fermentation today including organic acids and alcohols. Limitations in the further development of such processes are the cost and availability of feed-stocks as well as the genetic engineering of the cells (Straathof et al., 2005). Ultimately energy input, product yield on substrate, space-time-yield and product concentration will determine the economics of such processes.

4.2. *Industrial biocatalytic processes*

Bioprocesses based on the use of one or more enzymes as a catalyst, which may be in non-growing cells (often referred to as 'resting' cells) or supported on an 'immobilization' matrix or soluble in solution are known as biocatalytic processes (Schoemaker et al., 2003). Such processes are most prevalent today in the pharmaceutical industry (Pollard and Woodley, 2007) but also have a major impact in many other areas of chemical production, such as oils and fats processing (Schmid et al., 2001). Around 200 processes alone are already implemented in the pharmaceutical industry. Some of the issues surrounding industrial application of biocatalytic processes are summarized in Table 3. Limitations in the further development of such processes are the cost of downstream processing as well as the protein engineering of the enzymes. Ultimately product yield on substrate, product yield on enzyme, space-time-yield and product concentration determine the economics of such processes.

4.3. *Downstream processing*

The recovery of chemical products from fermentation and microbial-based or enzyme-based biocatalysis is often expensive and difficult. The primary reason is the dilute nature of the product stream. For a commercial process, concentrations around 100 g/L are required for high value products and around 300–400 g/L for bulk chemicals. Such concentrations are far away from those found in nature and although some biocatalyst engineering solutions may help, the low concentration is frequently the result of the inhibitory or toxic effects of products or co-products (or the dilute nature of the catalyst preparation). A potential solution developed over the last thirty years is *in situ* product removal (ISPR) (or *in situ* by-product removal – ISBR), where the product (or by-product) is removed from the site of reaction as is it formed (see Fig. 2). A variety of techniques and technologies are available today (Freeman et al., 1993; Stark and von Stockar, 2003; Woodley et al., 2008). It seems likely that such technology will be further developed in the future. Process integration of this type, where unit operations are combined has many complexities for biotechnological processes, but if mastered will enable many lower value products to be manufactured using biotechnological means. One of the most important new technologies for industrial biotechnology involves the membrane based unit-operations. Such operations are required especially for the compartmentalization and/or recycle of enzyme or cells. In many cases contactors to enable liquid-liquid extraction are also possible, also via in situ product removal (Pabby and Sastre, 2013).

5. Future bioprocesses

5.1. *Process integration with chemical operations*

It is clear that for the future it will be necessary to integrate bioprocesses into existing chemical plants. Today the cost of many chemical plants is already written-off, so replacement is not an easy option. This is particularly true for large plants of at least €500 million investment. In stages it seems likely that (1) capacity increases will be absorbed by bioprocesses and later (2) retrofit of existing processes will be carried out. At first a significant majority of steps will remain chemically based and later bioprocesses will expand into the majority if not the entire process. Finally it is clear that bioprocesses which operate under mild conditions (neutral pH, atmospheric pressure and ambient temperature) will enable options for process plant made from cheaper materials of construction. In some areas of bioprocessing disposable plant and polypropylene based tanks and pipes are already being used. Clearly this will have important consequences for plant design and investment of capital.

5.2. *Next generation biocatalysts*

Biocatalysts of the future will be far more sophisticated than today. For example rather than using a single enzyme for a conversion, multiple enzymes will be used in entirely new pathways or routes (Santacoloma et al., 2011; Sagt, 2013; Gardner, 2013; Xue and Woodley, 2012). Already today such an approach is in use for in situ cofactor regeneration. Such a

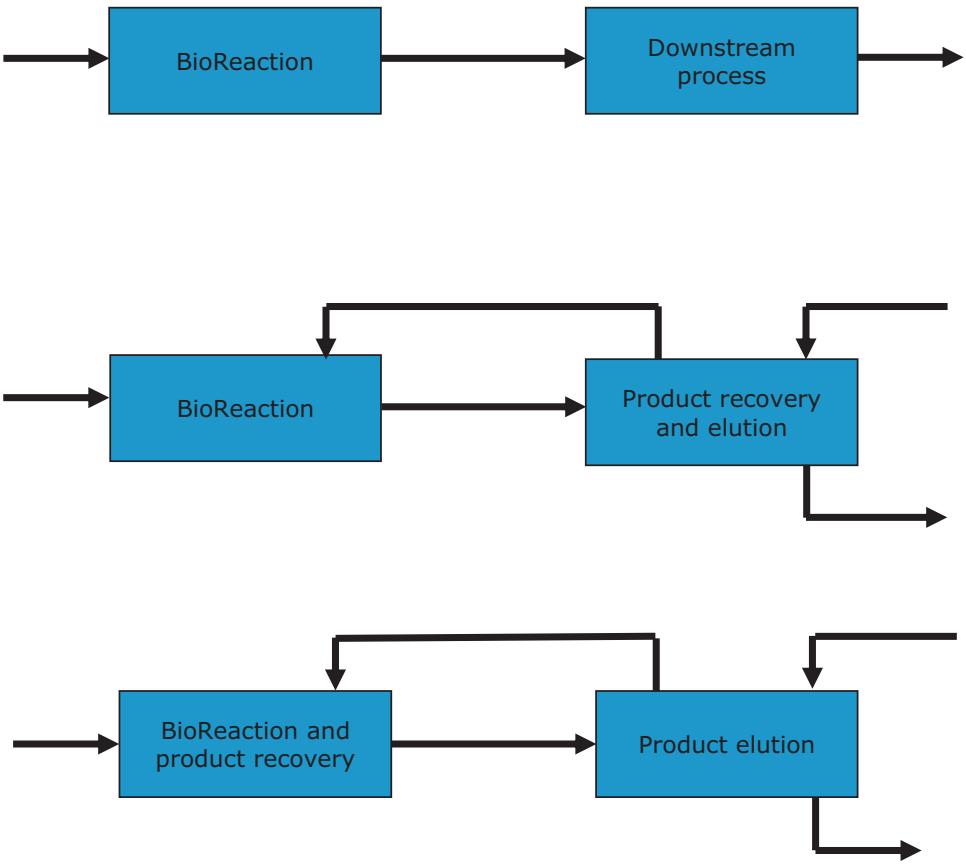

Fig. 2 – Schematic ISPR options.

concept builds on what nature already provides today (Bruggink et al., 2003). While this can provide inspiration, new pathways are required to build the necessary products of the future and attention will need to be paid not only to kinetics but also the thermodynamics of such de novo pathways. On occasion there will be value in operating the pathways outside cells and on other occasions inside cells (Rollié et al., 2012). In both cases the yield will be required to be focused on the desired product, with the aim of reducing intermediate separation and losses to by-products. Likewise protein engineering (Lutz, 2010; Bornscheuer et al., 2012) will enable alteration to biocatalysts such that designer catalysts will become possible (potentially enabling new chemistries), ultimately ensuring integrated operation in one pot, eliminating entirely intermediate separation and in some cases linked to neighboring chemical reaction steps (Woodley, 2013; Wei, 1996). Finally, there are already a few precedents where enzymes have not just been modified, but also designed from scratch capable of catalyzing reaction types which have not been found in vivo (Röthlisberger et al., 2008; Siegel et al., 2010)

6. Future engineering tools

6.1. *Rapid design methods*

Biology does not provide all the necessary solutions for industrial process challenges. The last decades have clearly shown the need for innovative process engineering solutions as well as highly sophisticated molecular biology to engineer the biocatalyst. Indeed it is highly likely integration of catalyst design and process design is part of the next paradigm in chemical engineering (Vennestrøm et al., 2010). Particularly interesting is that neither a single objective (e.g. lowest production cost or lowest development cost), nor development route (e.g. protein engineering or process engineering) nor solution (e.g. enzyme immobilization for 100 recycles with microfiltration or 5 recycles with soluble enzyme by ultrafiltration) exist, even in a given case. An excellent recent industrial example from the pharmaceutical industry is the use of an engineered ω-transaminase (EC 2.6.1.18) for the manufacture of Sitagliptin by Merck (USA), which was solved by integrated process and biocatalyst engineering (Savile et al., 2010; Truppo et al., 2012). In other sectors of industrial biotechnology the economic leeway for process optimization might be more limited than in the pharmaceutical business, but there remains room for improvement in many cases.

Frequently multiple strategies are required to come to a satisfactory economic solution. A clear need from the perspective of process engineers is to develop a means to navigate the solution space in an effective way. In the pharmaceutical sector the time limitations (as a result of a defined patent life) mean that the emphasis is on speed of development. It is clear that automated, systematic methods of data collection, linked with design of experiments and process models will have huge benefits in much the way they have already for in other sectors of the chemical industry.

6.2. *Process modeling*

Process modeling is increasingly implemented as a means of mathematically describing bioprocesses. It is of course easiest for enzyme-based bio-catalysis (Vasić-Rački et al., 2011), but is also necessary for complex fermentations where population based models are required. Two types of models need to be developed – those that describe the reaction phase and those that describe the downstream unit operations. Much progress has already been made but more sophisticated models are required to enable a more predictive approach for scale-up and design. This will also be an important contribution from chemical engineers in the future as we move from empirical to more mechanistically based models. Alongside this it will be necessary to build property databases of suitable feedstocks, reagents, and chemicals. In many cases predictive tools for the properties of many of these molecules would perhaps be even more useful, to save valuable experimental time. The chemistry, in particular of many processes where biological catalysts can best be exploited, is complex and the building of a suitable database and predictive tools will be an important contribution.

7. Future perspectives

There is little doubt that industrial biotechnology will expand as an important area of chemical engineering for the future. However this also represents a challenge both for academia and also industry, where change from the *status quo* will be required.

- *Changing academia*. In academia the challenge is how to educate chemical engineers in industrial biotechnology. Already today the curriculum is filled so full covering the basic scientific background and the special principles of chemical engineering as well as design. Many degree programs also offer extra courses that enable some degree of specialization from polymer technology to pharmaceutical processing. So one can easily conclude that there seems little room in the existing curriculum for teaching about bioprocesses and it is clear that master's programs focusing on this area are required. Many universities already offer such programs although here too the curriculum can become filled with the enormous diversity of bioprocesses from protein processing to small molecule processing. So even here some specialization may be required. The support of industry in enabling exposure to real industrial problems (via work experience or even case studies) is also important, but will require significant commitment. A final issue is that the very rapid development of the discipline has led also to a shortage of definitive texts for use as a support for students. Making biology quantitative will also require special care both from those teaching and those being taught.
- *Changing industry*. Much of the chemical industry today is under pressure. The need to innovate is one solution but it is still not straightforward. For example, in order to innovate via introduction of bioprocesses, various companies have tried different approaches ranging from specialist in-house teams to outsourcing, but in the end integration into the mainstream of a company from managers to design engineers means that new approaches are required including much more active continuing education programs to help and assist industry. To ensure success in the future, close collaboration, which is already a hallmark of industrial biotechnology today, will be essential between chemical engineers, chemists and biologists, preferably in a single physical location.

8. Concluding remarks

The last sixty years have seen enormous progress in the development of industrial biotechnology for chemical production and today many companies using such processes in an effective way to produce chemicals. Indeed today we can state that it is no longer a new technology but one that is taking a mainstream role in many research and development organizations in industry. Nevertheless education of the next generation of engineers and the continuing education of those already working in industry is also required. In such developments Europe has a particularly strong position and partly this is as a result of supportive funding for research from the EC and partly the result of excellent collaboration between industry and academia. This not only represents an exciting development but also helps all those in university to understand industrial needs and for industry to pick the very latest developments from academia. Such a synergistic relationship has served bioprocessing very well in the past six decades and there is little doubt it will continue to do so in the next six as well.

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