CHIMIA 51 (1997) Nr. 6 (Juni)

Chimia 51 (1997) 287–289

ISSN 0009–4293

Biotransformations for Fine Chemical Production

Hans-Peter Meyer*, **Andreas Kiener**, **René Imwinkelried**, and **Nicholas Shaw**

Abstract. Biotechnology has become an indispensable tool for the production of fine chemicals. The choice of route, chemical or biotechnological, for the manufacture of a given fine chemical is crucial. In general terms, biotechnology is the method of choice for large molecules with a high degree of functionalisation and multiple stereocentres. Most of *LONZA*'s biotechnological bioprocesses for the production of fine chemicals are whole cell processes using microorganisms which form very specific enzymes. Process improvement at *LONZA* is discussed in this paper on three levels: upstream processing, biotransformation/biosynthesis, and downsteam processing.

Introduction

The pharmaceutical industry is changing for a number of reasons, one of which is the emergence of new drugs produced by biotechnological means. The growth rate for these biopharmaceuticals, such as therapeutic proteins, peptides, antisense drugs, and monoclonal antibodies is higher than for classical, chemically synthesised drugs. However, this trend could be reversed and chemically synthesised drugs will definitely continue to play an important role.

Fifteen of the top-selling 25 drugs in 1994 were single isomers, and the trend towards chiral pharmaceuticals and agrochemicals is increasing. The total market for chiral synthons is estimated to be USD 250–300 Mio. and is expected to grow to USD 1 Bil. by the year 2000. Biocatalysis will play an increasingly important role in this area, because enzymes are selective and chiral, and a synthetic route that incorporates one or more biocatalytic steps is often more efficient than a totally chemical route.

LONZA has a long tradition of manufacturing fine chemicals for the life science industries by chemical synthesis. Since starting a small biotechnology research group 12 years ago, our company has successfully developed a number of processes incorporating one or more biocatalytic steps for the manufacture of fine chemicals [1–4]. This innovative approach has been furthered by the recent acquisition of *Celltech Biologics* (now *LONZA Biologics*), extending *LONZA*'s expertise to the production of monoclonal antibodies and therapeutic proteins.

Biotechnology and Chemistry are Complementary Technologies

The choice of route for the manufacture of a given fine chemical is crucial. Each target molecule must undergo careful retro-synthetic analysis, and in many cases, the result is that a combination of chemistry and biotechnology will result in the most efficient process. Often, a final decision can be made only on the basis of comparative laboratory experiments.

Synthetic organic chemistry offers a number of advantages including considerable experience at both laboratory and industrial scales, predictability and reliability, alternative reagents and reactions for given transformations, rapid construction of carbon skeletons, and insertion of basic functional groups. Progress can also be expected in asymmetric synthesis, especially in the field of transition-metal catalysis, which has not been fully exploited. However, organic synthesis also has a number of weaknesses, especially when it comes to functional group transformation in multifunctional molecules, functionalisation of nonactivated carbon atoms, and construction of complex, high-molecular-weight molecules with multiple stereogenic centres. Fortunately, biotechnology can complement organic chemistry to overcome some of these weaknesses. Biotechnological manufacturing processes are especially useful for larger molecules with a high degree of functionalisation, and the development of powerful new biocatalysts is resulting in important progress in the field of chemo-, regio-, and stereoselective transformation. The production of enantiomerically pure compounds bearing chiral hydroxy, carboxy, amino, and sulfoxide groups are of particular commercial importance (*Table 1*), and research for biocatalysts for this area will retain a high priority. However, the potential of enzymes for biotransformations has not yet been fully exploited. According to *Faber* [6], of the 2500 known enzymes, 250 are used commercially in more or less purified forms, and merely 25 account for over 80% of all commercial applications (*ca.* USD 500 Mio. in 1990) mainly for food and starch processing and as detergent additives. For biotransformations, the hydrolytic enzymes such as lipases and esterases have been used, because they are available in commercial quantities, often have broad substrate spectra, and are usually simple, robust, have no cofactor requirements and are therefore easy to use (*Fig. 1*). The future use of other enzyme types, *e.g.* lyases and reductases, will be driven by commercial requirements and will depend on advances in such areas as cofactor recycling and protein engineering.

Even though the selectivity of enzyme-catalysed reactions often exceeds those of chemical reactions, it does not necessarily mean that the biological approach is more efficient. Other criteria, such as the availability of starting materials and product recovery, can be critical. For a company to be successful in the fine chemicals business, expertise in both organic synthesis

Table 1. *The Occurrence of Functional Groups at Chiral Centres in Pharmaceuticals* [5]

	Functional group	Occurrence (as % of chiral centres)
Hydroxy	–OH	40
Carboxy	–COOH	22
Amine	$-NH_2$	16
Sulfoxide	$>S=O$	3
Other		19

**Correspondence*: Dr. H.-P. Meyer
Department of Biotechnology
LONZA AG
CH-3930 Visp

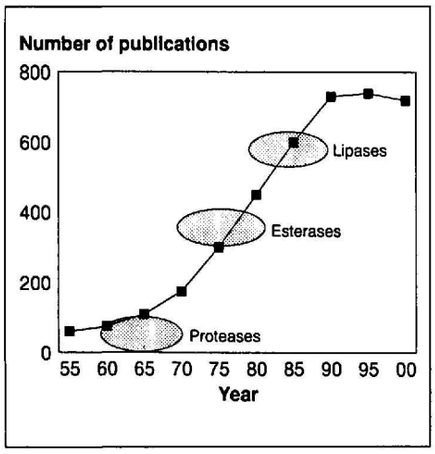


Fig. 1. *Number of publications per year in the area of biotransformations.* The graph shows that the number of publications has reached a steady state. Many papers on hydrolytic enzymes, especially proteases, esterases, and lipases were published.

and biotechnology is essential. *LONZA* is proud to be in this position.

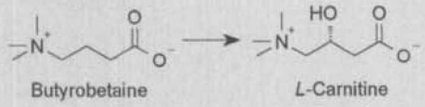
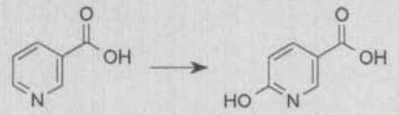
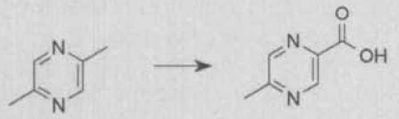
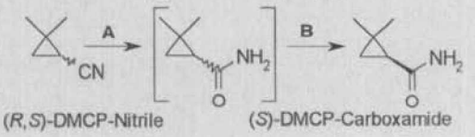
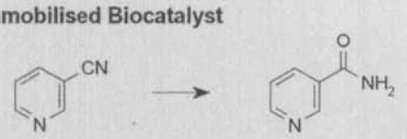
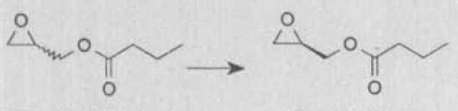
Care of the environment is also important, and in some cases bioprocesses produce less waste than chemical routes. A comparison of the waste stream from the *LONZA* L-carnitine bioprocess [7] with that from the *LONZA* chemical synthesis [8] is shown in *Fig. 2.*

Whole Cell Processes

Biosynthesis is the production of a substance *de novo* by living cells, and the production of a secondary metabolite would be a typical example. Biotransformation is the conversion of one substance into another, catalysed either by whole cells or by isolated enzymes. The starting material can be synthetic or natural. Cells for biosynthesis and biotransformation are produced by fermentation. Fermentation and biotransformation can take place at the same time (precursor fermentation), as *e.g.* with 5-methylpyrazine-2-carboxylic acid (MPCA), or separately, as with 6-hydroxynicotinic acid (*Table 2*).

The only industrial scale biotransformation that *LONZA* carries out with a commercially available enzyme is the production of the chiral intermediate (*R*)-glycidyl butyrate by resolution of the racemic compound with porcine pancreas lipase (*Table 2*). The reason that commercial enzymes are not used more often is that they are not always suitable for a specific biotransformation. Consequently, microbial strains with the desired activity must be found, either by screening or selection. Most of the bioprocesses developed by *LONZA* for secondary metabolites, vitamins, steroids, and intermediates therefore rely on whole cells and make use of their high selectivity and productivity. *E.g.*, the regioselective functionalisation of nicotinic acid by purely chemical methods is difficult to control, and therefore the hydroxylation of nicotinic acid to 6-hydroxynicotinic acid by *Achromobacter xyloxidans* opens up a novel route for the production of substituted nicotinic-acid derivatives. The biotransformation of 3-cyanopyridine illustrates the use of an immobilised biocatalyst by *LONZA* for the continuous multistage production of 3000 t of nicotinamide per year.

Table 2. *Commercial Biotransformations Developed at* LONZA *Using Various Reaction Types and Biocatalysts*

Biotransformation	Enzyme class	Biocatalyst
Whole Cell		
 Butyrobetaine → *L*-Carnitine	dehydrogenase hydratase	*Agrobacterium* HK 1349
 Nicotinic acid → 6-Hydroxynicotinic acid	'hydroxylase'	*Archromobacter xyloxidans*
 2,5-Dimethylpyrazine (DMPY) → 5-Methylpyrazine-2-carboxylic acid (MPCA)	monooxygenase 2 dehydrogenases	*Pseudomonas putida*
 (*R,S*)-DMCP-Nitrile → (*S*)-DMCP-Carboxamide	A) nitrile-hydratase B) amidase	A) *Rhodococcus equi* B) *Escherichia coli* DH5/pCAR6
Immobilised Biocatalyst		
 3-Cyanopyridine → Nicotinamide	nitrile-hydratase	*Rhodococcus rhodochrous* J1 (Nitto)
Isolated Enzyme		
 (*R,S*)-Glycidylbutyrate → (*R*)-Glycidylbutyrate	lipase	Pig Pancreas Lipase

Strain Selection

A strain suitable for a specific biotransformation may be found by screening, selection, and classical mutagenesis, and then genetic engineering techniques can be used to overexpress a single enzyme or a whole pathway [9]. The strategy depends on the complexity and economics of the process, the time available, and the market volume of the final product. A first generation process will often be put into place, followed by a second generation process based on an improved biocatalyst. *E.g.*, (+)-(*S*)-2,2-dimethylcyclopropanecarboxamide is an intermediate for the synthesis of the dehydropeptidase inhibitor Cilastatin, and *LONZA* has developed a process which involves the chemical synthesis of the racemic nitrile, followed by a two-step biotransformation. The *Comomonas acidivorans* strain with the enantiospecific amidase was found by selection, and then the specific biotransformation rate was improved 20-fold by cloning the amidase gene into *E. coli* [3].

CHIMIA 51 (1997) Nr. 6 (Juni)

Process Improvement

Process improvement at *LONZA* is focussed on three areas. The first is medium and inoculum preparation, because the use of complex and undefined raw materials results in dilute product solutions containing heterogeneous impurities [10] and leads to high product recovery costs. The second is fermentation and biotransformation. Here biological regulation mechanisms play an important role. Substrate and product inhibition, the stability of strains and enzymes, growth factor and cofactor requirements, and by-product formation all have to be considered. Because commercial processes have to be developed quickly, and understanding biological control mechanisms can be a slow process, adapting existing process technology to overcome problems in this area is a particular challenge. The biotransformation of 2,5-dimethylpyrazine (DMPY) to 5-methylpyrazine-2-carboxylic acid is an example. The starting material, the product, and the carbon source (xylene) used for fermentation all inhibit the process. Flow injection analysis (FIA) for online control, modelling, and expert systems combined with computer control allow complicated feeding strategies to overcome the substrate inhibition [11]. Downstream processing (DSP), or product recovery, is the third area for process improvement. Again, the application of process engineering may help to overcome problems such as product inhibition, and *LONZA* is currently evaluating different methods based on liquid-liquid extraction, chromatography and membrane processes [12][13]. However, the selective, continuous, and sterile removal of an inhibiting product during a biotransformation (*in situ* product recovery, ISPR) is difficult. Large-scale liquid-liquid extraction and simultaneous back-extraction, with *e.g.* quarternary amines, is difficult because of low k_La values. The use of membranes for perstraction will improve liquid-liquid extraction methods if the ratio of membrane surface to the volume of reactor liquid can be reduced. Methods such as electrodialysis and nanofiltration can sometimes be used for ISPR and product isolation and purification, but currently immobilisation of the inhibiting product on an ion-exchange matrix has shown the most promise for the ISPR of nonvolatile products at *LONZA*. *Fig. 3* shows an example of a schematic layout for the continuous removal of an inhibiting product by ISPR, and a possible recovery process for the dissolved product.

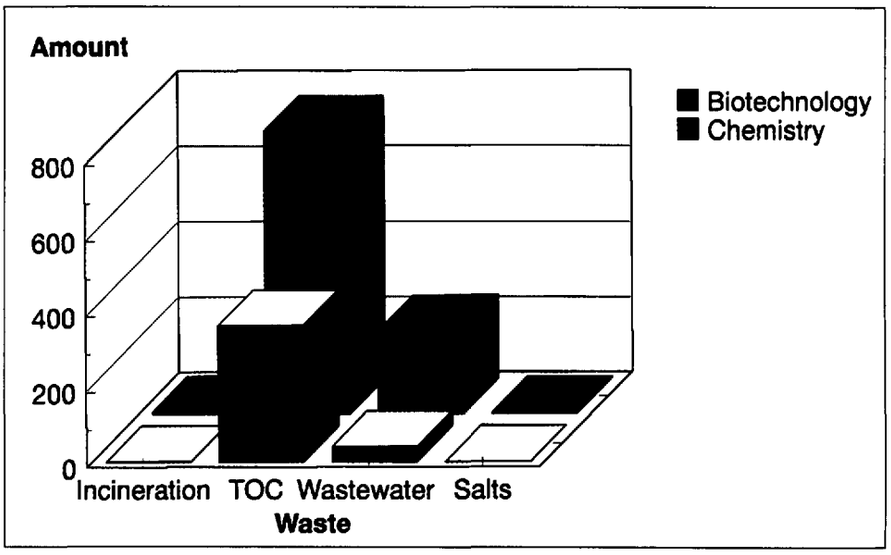


Fig. 2. *Comparison of waste generated by chemical and biotransformation processes for the production of* L-*carnitine.* The main differences are the wastewater in m^3 per ton of product and the total organic carbon (TOC). Both values are much lower in the biotransformation process. Waste salts and waste for incineration in tons per ton of product are low for both processes, with the values for the biotransformation process being 3–6 times lower than those for the chemical process.

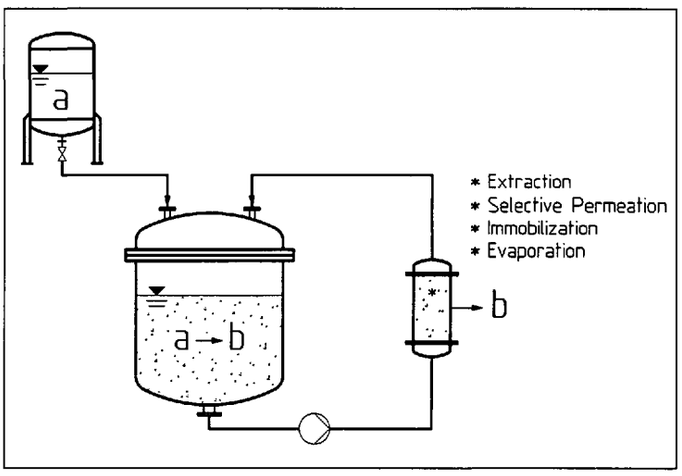


Fig. 3. *Schematic layout for the continuous removal of an inhibiting product b by ISPR* (in situ *product recovery) and a possible recovery process for the dissolved product*

Biocatalyst and enzyme immobilisation, biocatalysis in organic solvents, process integration, bioreactor design, chemostat systems for manufacturing [14] and the optimisation of growth media and other parameters are other areas that *LONZA* has identified for future investigation.

Biotechnology has become an indispensable tool for the manufacture of fine chemicals, and its importance in this field will increase.

Received: April 18, 1997

[1] H. Kulla, *Chimia* **1991**, *45*, 81.
[2] A. Kiener, *Chemtec* **1995**, *25*, (No. 9), 31.
[3] O. Birch, J. Brass, A. Kiener, K. Robins, D. Schmidhalter, N. Shaw, T. Zimmermann, *Chim. Oggi* **1995**, September, 9.
[4] H.-P. Meyer, *Chimia* **1995**, *47*, 59.
[5] Personal communication, S. Taylor, *Zeneca* Bioproducts.
[6] K. Faber, 'Biotransformations in Organic Chemistry', Springer Verlag, Berlin, 1995.
[7] T.P. Zimmermann, K.T. Robins, J. Werlen, F.W.J.M.M. Hoeks, 'Biotransformation in the Production of L-Carnitine' in 'Chirality in Industry II', Eds. A.N. Collins, G.N. Sheldrake, and J. Crosby, Vol. 2, John Wiley, Chichester, 1997, p. 287.
[8] R. Voeffray, J.-C. Perlberger, L. Tenud, J. Gosteli, *Helv. Chim. Acta* **1987**, *70*, 2058.
[9] J.S. Dordick (Ed), 'Biocatalysis for Industry', Plenum Press, New York, 1991.
[10] H.-P. Meyer, *Chimia* **1994**, *48*, 455.
[11] M. Rohner, H.-P. Meyer, *Bioprocess Engineering* **1995**, *13*, 69.
[12] A. Jaquet, I.W. Marison, H.-P. Meyer, U. von Stockar, *Chimia* **1996**, *50*, 426.
[13] R. Glöckler, J.-P. Roduit, *Chimia* **1996**, *50*, 413.
[14] F.W.J.M.M. Hoeks, H. Kulla, H.-P. Meyer, *J. Biotechnology* **1992**, *22*, 117.