possible to be economically competitive with other bulk surfactants.

It may be of interest for scientists concerned with enzymatic synthesis to learn about the obstacles met during upscaling of this process. Surfactants are bulk chemicals. The size of the current market in the EEC, just for non-ionic surfactants, is in the order of 700000 t per year. At an estimated average price of 1.5 US$/kg active substance the EC market thus amounts to 1 bio US$ p.a. The economic condition mentioned above is thus fulfilled, even if this market is made up of maybe hundreds of different chemical species.

If a reasonable share of this market is aimed for, it is necessary that the raw materials are available in sufficient quantity. This reprents the first obstacle. The basic raw materials are fatty acid, glucose and ethanol all of which are available in sufficient quantities, or are they? Ethanol is OK, but what about the glucose? The process as developed in the laboratory uses anhydrous glucose, and besides being expensive, compared to glucose syrup, it is a product which is gradually disappearing from the market. It owed its market to the fact that historically it was the purest glucose available, therefore, it was prescribed in the various pharmacopeias. Today, it is possible to get just as pure glucose monohydrate which is cheaper, and as the pharmacopeias are updated, anhydrous glucose disappears in the prescriptions, and thereby the main market for anhydrous glucose vanishes. As a consequence, plants are being closed down and, in the future, the amounts necessary for production of, say, 20–30 000 t.p.a. of surfactants will certainly not be available.

Now to the fatty acid. For marketing and for purity reasons, pure fatty acids of non-petrochemical origin are preferred. Of some of these there is enough, but some, like myristic acid and, to a certain extent, capric acid are only available in quantities which can not form the basis of the aforementioned production figures.

Another important raw material is the carrier for the immobilized enzyme. In the development work, it turned out that only one of many commercially available carriers allowed for the necessary number of re-uses. This carrier, besides being expensive, varies from batch to batch in a non-predictable way as regards immobilization capacity.

Looking to the production process, several problems occurred which were not a problem in laboratory scale. Even if these problems are only partly related to enzyme application, they should be mentioned here.

The first reaction step, the conversion of glucose to ethyl glucoside is catalyzed by an ion-exchange resin. This leads to the formation of a small amount of diethyl ether (*ca.* 1%). Because the reaction is conducted with a large surplus of ethanol, and this ethanol is to be recirculated, a build-up of ether will occur leading to risk of explosion.

Because the enzyme is unstable towards heat, the reaction temperature during esterification may not exceed 70°, if the necessary number of re-uses shall be obtained. This has two consequences, one is a relatively long reaction time which is costly, and the other is that viscosity becomes a problem, especially with the long-chained fatty acids, *e.g.* palmitic and stearic acid.

Finally, it should be mentioned that going from laboratory scale to a reactor size of several tonnes means longer heating and cooling time with increased colour as a consequence.

Despite all these difficulties, the process moves towards commercialization. If it succeeds, it will show the industry that it is possible to produce bulk chemicals with the aid of enzymes.

[1] Figures calculated from the following sources (amounts are for 1984): *Chem. Eng. News*, May 6 **1985**, 13; *Chem. Marketing Rep.*, **1992**, 242, (22).

[2] O. Andresen, 'Proceedings of BioTech '85 USA', Washington, D.C., October 1985, p. 253.

[3] a) O. Kirk, F. Björkling, S.E. Godtfredsen, to *Novo Nordisk A/S*, 1989, Int. Pat. Appl. WO 89/01480; b) F. Björkling, S.E. Godtfredsen, O. Kirk, *J. Chem. Soc., Chem. Commun.* **1989**, 934; c) K. Adelhorst, F. Björkling, S.E. Godtfredsen, O. Kirk, *Synthesis*, **1990** *2*, 112; d) O. Kirk, F. Björkling, S.E. Godtfredsen, to *Novo Nordisk A/S*, 1990, Int. Pat. Appl. WO 90/09451.

Chimia 47 (1993) 123–126

ISSN 0009–4293

Fine Chemicals: From Research to Production

Hans-Peter Meyer*

1. Introduction

Fine chemicals are produced in limited quantity by a limited number of manufacturers. They are often used exclusively for the preparation of one specific drug or agrochemical. Unlike performance or specialty chemicals, they are sold usually according to specifications – according to what they are [1]. At *Lonza AG*, biotechnology is used to complement organic chemistry in the production of fine chemicals, usually optically active pharmaceutical or agrochemical intermediates (*Scheme*). Due to a shortage of large fermentors between 1986 and 1991, we successfully adapted a chemical plant to carry out full scale fed-batch fermentations and biotransformations [2]. Since 1992, we have acquired a production plant in Czechoslovakia with 15 m^3 and 50 m^3 fermentors, which were adapted to our specific needs, and where we now manufacture our fine chemicals. This paper will discuss the practical problems that we encountered when scaling up and transferring technologies from research to production.

2. The Whole Cell Bioprocess

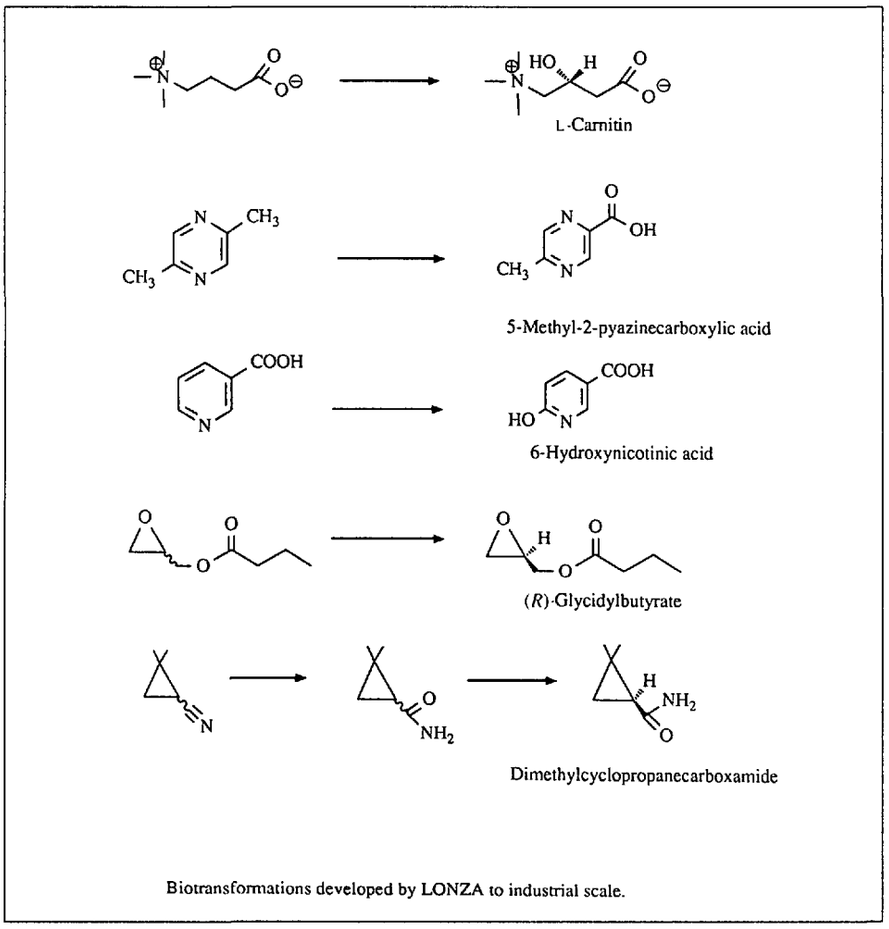
We have scaled five processes up to production following the general process scheme depicted in *Fig. 1*. Four of the processes are whole cell processes, using different bacterial species, modified by recombinant DNA technology or by classic genetic methods. It demonstrates, how fermentation and biotransformation are integrated into one manufacturing process consisting of many different steps, including the chemical synthesis of the educt for biotransformation. *Fig. 1* shows also the two countercurrent flows, of material on one hand, and the customers requests such as specification and cost on the other hand.

**Correspondence*: Dr. H.-P. Meyer
Lonza AG
CH–3909 Visp, Switzerland

The first example in the *Scheme* is the biotransformation of chemically synthesized γ-butyrobetaine into optically pure L-carnitine using a mutant strain. Fermentation and biotransformation are carried out simultaneously in one bioreactor as a fed-batch process. Using another organism, able to degrade organic solvents such as xylene or toluene, chemically synthesized dimethylpyrazine is oxidized into 5-methyl-2-pyrazinecarboxylic acid, in a one pot fed-batch process similar to L-carnitine. For 6-hydroxynicotinic acid, chemically synthesized nicotinic acid undergoes a site-specific hydroxylation by an aerobic organism (*Scheme*). However, biomass production and biotransformation are carried out sequentially, the first as fed-batch fermentation followed by a batch biotransformation of nicotinic acid to 6-hydroxynicotinic acid. All fed-batch processes have one thing in common: several feed streams (typically 2–3) must be varied according to an exact pattern, which must be controlled accurately. The remaining two examples in the *Scheme* are both racemic conversions resulting in optically pure synthons. For (*R*)-glycidylbutyrate, we are using a commercially available lipase [3], which hydrolyses the racemate, produced by in-house chemical synthesis starting from glycidol and butyrylchloride. For the production of the dimethylcyclopropanecarboxamide, however, we produce the active biomass again in-house. The biomass production with hydratase activity is a fed-batch process, whereas the biomass production for amidase is a relatively simple batch fermentation.

Two processes, L-carnitine [4] and 6-hydroxynicotinic acid were also realized as chemostat processes in the 450 l scale. However, despite considerably higher volumetric productivity of the chemostat process, we use exclusively fed-batch operation because of the overall simplicity and reliability of the discontinuous process. The aspect of reliability and reproducibility is particularly important because of the difficulties of isolation and purification.

Scheme



Biotransformations developed by LONZA to industrial scale.

3. The Relation between Biology and Chemistry

A representative isolation and purification for one of our fine chemicals, L-carnitine was described by *Hoeks* and *Mühle* [5]. Our experience with L-carnitine as well as with other fine chemicals has taught us that for an economically and ecologically sound production process, the decisive step is the chemical isolation and purification of the product after the biotransformation. Generally the cost for the down stream processing (DSP) also represents the major portion of the manufacturing costs with fine chemicals. However, the simplicity of the product isolation and purification process is exclusively dependent upon the fermentation. The ideal result of fermentation and biotransformation is a cell free, aqueous product solution, virtually free of impurities with

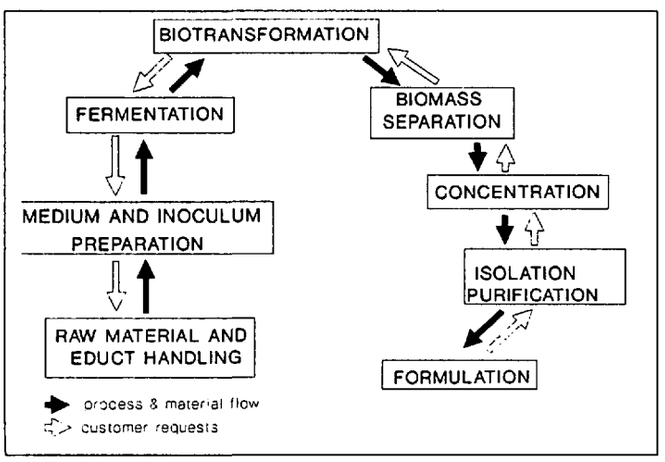


Fig. 1. *Flow scheme of a whole cell bioprocess*

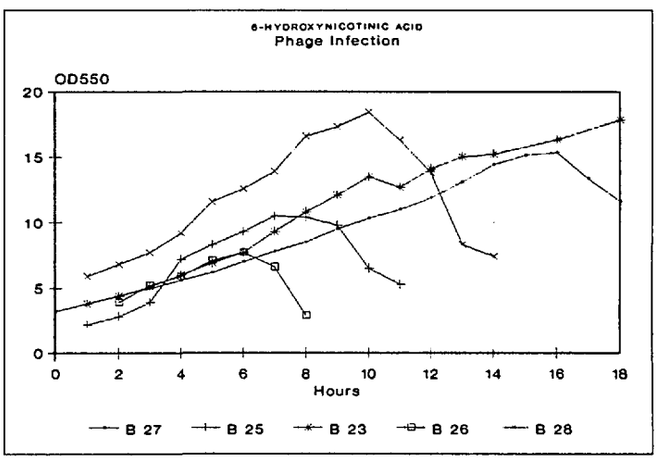


Fig. 2. *Effect of phage infections*

high product concentrations, preferentially over 10% (*w/v*). Furthermore, isolation and purification in an aqueous environment is an advantage.

Table 1 concentrates on the important problems encountered during the fermentation/biotransformation step, which consequently makes isolation and purification more costly and difficult. Generally speaking, all problems can be related to the design of the bioreactor, specifically in relation to sterility and to mixing.

3.1. *Sterility*

For example unsterile conditions often cause coloured end products, although productivity and yields are at their expected level. The effects of phage infections, which are by the way dependent on the time of the year, were disasterous with respect to both quality and quantity. *Fig. 2* shows the effect of a phage infection during the active biomass production for 6-hydroxynicotinic acid in the 12 m^3 scale. Four affected batches are compared to a noninfected batch B 23.

The problem of infections not only arises during fermentation, but can also be an impediment during any step where products or educts are stored *e.g.* as solution. The cell free L-carnitine solutions (10% *w/v*) from fermentation are vulnerable to about any infectant. In the case of 6-hydroxynicotinic acid, we even had fungal infections in a final crystal product, which was not immediately dried after centrifugation.

3.2. *Mixing*

The energy that is introduced into a fermentor by the sparger and the stirrer is dissipated by a combination of fluid mass flow and the level of turbulence (see above) contained in the flow. Mixing is important with respect to homogenization on one hand and transfer of oxygen by dispergation on the other hand. Since most of our processes are aerobic bioprocesses, oxygen transfer rates (OTR) have a direct influence on the volumetric productivity. Although macromixing was found to be more important in our case, volumetric productivity and OTR were nevertheless optimized in the possible limits. More critical was the improvement of mixing for optimal fluid mass flow, which was required for the homogenization of fluid elements (educts) with the bulk of the fluid in the fermenter. Imperfect mixing leads to gradients within the reactor. As mentioned before, our fed batch processes follow stringent feed regimes, and the tolerance for deviation from the (local) optimal educt concentration can be small. Moreover, in the case of 5-methyl-2-pyrazinecarboxylic acid, we have to deal with a carbon source, with little water solubility and growth-inhibiting characteristics.

Table 1. *Some of the Problems Encountered when Scaling up a Biotransformation Process to Industrial Scale.* Most of those mentioned below affect and complicate the down-stream processing.

- Microbial contaminations during all phases of the manufacturing process.
- Phage contaminations with *Pseudomonas* and related strains.
- Poorly soluble educts or products and two-phase systems.
- Concentration gradients in the bioreactor.
- Limiting oxygen transfer rates.
- Optimal growth conditions differ from optimal product formation.
- Inadequate raw material and media composition.

Table 2. *Some of the Solutions Realized to Counteract the Problems of Scaling-up a Biotransformation for fine Chemicals to Industrial-Scale.*

- Integrative (team) approach from research to production.
- Think about isolation and purification right from start.
- Simulating large-scale early is recommended.
- Other technologies may offer solutions to biotechnological manufacturing problems.
- Reliable automation of a process can be extremely rewarding.
- Strain stability and reliability with respect to the industrial environment.
- Strain: fast growing, phage resistant, highly specific for extracellular product.
- Medium design: boring but important.
- Avoid unsterile processes whenever using living cells.

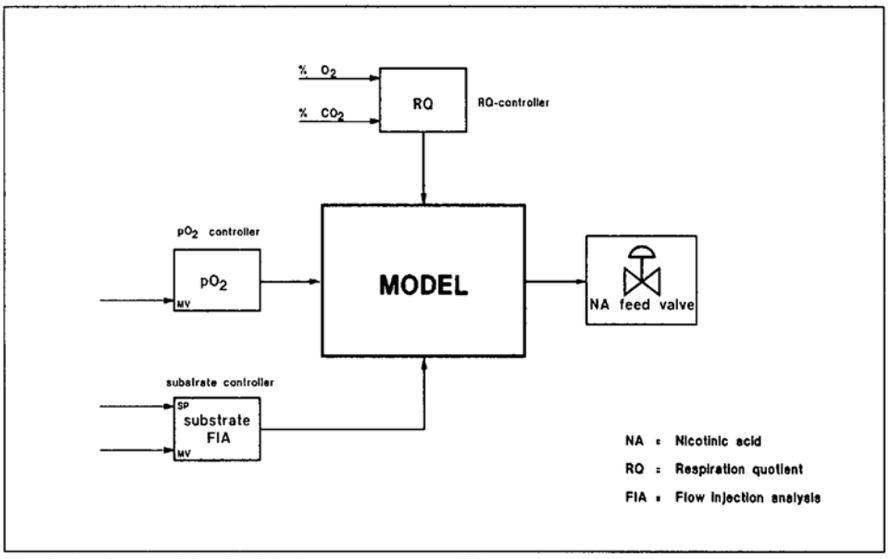


Fig. 3. *Feed control for the 6-hydroxynicotinic-acid fermentation*

4. Goals for Optimization of Fermentation and Biotransformation

A key factor to sucess of a biotechnologically-produced fine chemical is the cooperation of the different technical skills (research, development, production, quality) right from beginning of any project. A finely tuned and orchestrated team of chemists and biotechnologists will solve (almost) any technical problem. It is advisable to solve problems in a parallel way, and to make sure all interfaces are well organized. One first thing to do is obvious: optimize fermentation (*Table 2*). Optimizing fermentation normally means increasing productivity and yields. Often, however, the limits are set by unwanted by-products and/or incomplete biotransformation, increasing the operational effort in DSP. Thus, the biological maximum is often not identical with the optimum for the isolation and purification.

4.1. *Automation*

For two processes, 6-hydroxynicotinic [6] acid and 5-methyl-2-pyrazinecarboxylic acid, the on-line control of educts and products and subsequent automation of the process using a model algorithm was found to be an essential condition for successful manufacturing on a large scale. *Fig. 3* shows how we measure and control the process on-line in the case of 6-hy-

droxy-nicotinic acid. The case of 5-methyl-2-pyrazinecarboxylic acid is even more complex, where we developped a fully automated process with two interdependent feed streams based on a model using four process variables measured online. In both cases, the corresponding flow injection analysis and the realization of the process automation in production scale were developed in-house. The major advantages using this approach are qualitatively and quantitatively highly reproducible batches. One draw-back, however, is that the complicated hard and software needs highly trained operators, who are able to deal with any hardware problem.

4.2. *Stirrer and Sparger*

Standard bioreactors reach maximum oxygen transfer rates (OTR) of *ca.* 200 mmol/l/h, which were found to be largely sufficient for our processes. Moreover, in case oxygen transfer rates are limiting, they are only so during a short period of the fermentation, and, therefore, often do not justify the cost for adaptation. Nevertheless if an adaptation is vindicated, we considered increasing OTR either by improving turbulence and/or bubble size and distribution [7]. One approach was to use sintered steel spargers, that provide bubbles of the appropriate size. Another possibility is the use of injectors instead of spargers, which we have used for fermentations up to the 12 m^3 scale with very good results. Stirrer types with low power requirements but with high gas dispersion efficiency, *e.g.* an upward pumping pitched blade-stirrer or hollow blade agitator, have also been used. The use of antifoam, which by coalescing action lowers k_la values, should be kept to a strict minimum. Foam prevention, therefore, can be an effective and cheap way improving OTR values.

As mentioned above, we found the so-called macromixing or fluid mass flow to be more crucial. The distribution of a fed educt over the whole bioreactor volume in a short time, was achieved by a careful configuration of the right turbine (see above) in combination with multipoint feeding using dip pipes at the correct place in the bioreactor.

4.3. *Phage Prevention*

In the case of *Pseudomonas*, we must pay particular attention to the possibility of phage infections, which we encountered several times and in different geographical locations. There exist several technical options for prevention: active carbon, heat treatment, filtration by absolute filters or depth filters of the air. In our case we chose a depth filter, which is used in the electronic industries, for the phage decontamination of the sparging air for the fermenter. However, this physical measure is always accompanied by the availability of phage resistant mutants. To be on the safe side, we reduced the risk of recirculation of any phage contaminant by separating in- and out-flow of air and liquid. Since a production facility can hardly be relocated, the seasonal risks (airborne soil phages in dust) should be taken into consideration. Manufacturing with highly phage-sensitive production strains during the agriculturally active seasons of the year should be avoided.

4.4. *Sterility*

Although some of our fast processes such as 6-hydroxynicotinic acid or 5-methyl-2-pyrazinecarboxylic acid can and have been carried out under non-sterile conditions, it is not recommended due to quality fluctuations observed in the isolation and purification process. Therefore, the production of the active biomass for all processes are restarted from a sterile inoculum preferentially, for some examples a continuous (fill and draw) approach can be chosen.

4.5. *Product Storage*

Although some of our processes operate with organic solvents during the fermentation or biotransformation, the cell-free product solutions are always aqueous. Because of the easy infection and degradation of the cell-free product solutions, the isolation and purification, often preceded by a concentration step, should be carried out right after ultrafiltration. In case transport is necessary, this should take place with a concentrated product solution and the necessary precautions. In order to be able to concentrate, isolate and purify our products still in aqueous solution, we are using a number of methods wich are well established in fields other than biotechnology, such as electrodialysis, reverse osmosis, ion exchange and others. Especially membrane-assisted isolation and purification techniques offer some distinct advantages for aqueous product solutions.

5. The Future

The immediate challenge of a young business such as biotechnology in fine chemicals, is primarily its commercial survival in a competitive market. Two competitive forces have to be dealt with: competition from established producers on the commercial side and competition from chemical syntheses on the technical side. Research may be difficult, development and scale-up to manufacturing scale tedious, but rarely have ideas vanished because of purely technical reasons. The real problem is the identification of a good project idea, *i.e.* a real demand on the market place, plus an economic advantage of biotransformation *vs.* classic organic synthesis. Therefore, it seems advisable to find out, what established manufacturing biotechnologies could do for new product ideas from different corners. Besides microorganisms living in niches of the extreme, plant technology could be a source of interesting products and fine chemicals in particular. Technologies from biochemistry to mass culture of cells have been applied successfully with one exception: plant cell culture. In terms of technology, it is striking, how little plant cell culture is used in biotechnology especially for the bioreactor production of fine chemicals, with the exception of *e.g.* shikonin [8]. Also the potential ability of plant cells for biotransformations or synthetic purposes is probably underestimated. For example, bryophytes produce a remarkable variety of rare and new natural compounds, partly with striking biological activities or interesting structures [9][10]. A systematic screening of higher and lower plants and microalgae may reveal beneficial fine chemicals, building blocks and biologically active compounds. Meeting both ends, a market request for new and interesting fine chemicals and their production, it must be checked how the biotechnologies for mass production available today, must be varied for the industrial production with plant cell culture tomorrow.

[1] P. Pollak, *Chimica Oggi/Chemistry Today* **1992**, *10*, 11.
[2] H.-P. Meyer, *Biotech Forum Europe* **1991**, *8*, 602.
[3] H.-P. Meyer, J. Günther, M. Eyer, R. Voeffray, Proceedings, Chiral Synthesis Symposium and Workshop, Manchester (England), April 18, 1989, p. 31–33.
[4] F.W.J.M.M. Hoeks, H. Kulla, H.-P. Meyer, *J. Biotechnol.* **1992**, *22*, 117.
[5] F.W.J.M.M. Hoeks, J. Mühle, Extended Abstract, GVC/DECHAM Conference Wechselwirkungen zwischen Bioprozess und Aufarbeitung, Bamberg (Germany), Mai 25, 1991.
[6] M. Rohner M., H.-P. Meyer, Poster and Oral Presentation, EFB Workshop Bioreactor Engineering Course, Island of Arbarella (Italy), September 26–October 1, 1992.
[7] F.W.J.M.M. Hoeks, H.-P. Meyer, D. Quarroz, H. Helwig, P. Lehky, Extended Abstract, GVC/DECHEMA Conference Massstabvergrösserung in der Biotechnik, Lüneburg (Germany), Mai 6, 1991.
[8] V. Srinivasan, D.D.Y Ryu, *Biotechnol. Bioeng.* **1992**, *40*, 69.
[9] H.D. Zinsmeister H.D., R. Mues, *GIT Fachz. Lab.* **1987**, *6*, 499.
[10] A. Scheidegger, *TIBTECH* **1990**, *8*, 197.