Chimia 47 (1993) 122–123

ISSN 0009–4293

Production of Bulk Chemicals with the Use of Enzymes. Scope, Limitations, and Practical Examples

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Seen from an enzyme-producers view, synthesis of organic compounds with the aid of enzymes represents a potentially interesting new market. Having exhausted the obvious applications in traditional enzyme markets, *e.g.* starch hydrolysis and isomerization, cheese, beer, alcohol, fruit juice, bread, and, not least, detergents, the area of production of organic chemicals seemed an attractive opportunity.

This was the view at *Novo Industri* in the mid-seventies. In cooperation with DSM of the Netherlands, a screening was initiated for chemical reactions of industrial interest where the advantages of enzymes would have significant impact. More than twenty reactions were investigated ending up with focusing on nitrilases, specifically for production of caprolactam. There were several reasons for this. The initial chemical reason was the possibility of converting a nitrile to an amide, completely and without any formation of the corresponding carboxylic acid, using a purified nitrile hydratase. But the main reason was an economical one.

Development of an industrial enzyme is a costly affair. And with time it has become more and more costly, because the requirements of the authorities have been steadily increasing. The average development time for an industrial enzyme is around seven years, involving screening, yield improvement, safety testing, stabilization, often cloning, and immobilization development. As a rule of thumb, an enzyme should be able to yield an annual turn-over of minimum 5 million US$ in order to pay back the development cost. Applying another rule of thumb, *i.e.* that the enzyme costs are between 0.5 and 5% of the total production costs of a given product, and assuming an (optimistic) penetration of 10% of the world market, it follows that the target chemical must have a turn-over of 1–10 bio US$ p.a. As can be seen from the *Table*, the 25 largest organic chemicals just live up to the requirement (assuming world market to be around 3 times the US market). But looking at the list, it is difficult to find a promising candidate for enzymatic production.

This does not mean that the prospects are hopeless, however. There are several ways out:

Table. *Organic Chemicals among Top 50 Chemicals in the USA* [1]

Chemical	Sales [bio US$]
Ethylene	6.24
Ethylene oxide	2.68
Ethylene dichloride	2.33
Terephtalic acid	2.24
Propylene	2.17
Styrene	1.85
Ethylbenzene	1.46
Benzene	1.34
Formaldehyde	1.14
Propylene oxide	1.09
Vinyl chloride	1.05
Ethylene glycol	0.97
Adipic acid	0.90
Phenol	0.77
Vinyl acetate	0.77
Xylene	0.71
Acrylonitrile	0.66
Toluene	0.60
Acetone	0.57
Methanol	0.54
Cumene	0.54
p-Xylene	0.50
Butadiene	0.48
Isopropanol	0.41
Cyclohexane	0.34

- One may use an already commercial technical enzyme.
- An enzyme may be developed which can be used in many different production processes.
- The enzyme producer may decide to integrate forward and also produce the substance itself.
- Chemicals with a high added value may be identified which can carry a much higher enzyme cost.
- Processes may be identified where the fixed costs of production are very high which leaves room for a high enzyme cost, if fixed costs can be reduced proportionally.

Based on the second option above, it was decided to develop a nitrilase. In fact, several nitrilases were developed, both pure nitrile hydratases, which convert nitrile to amide, and pure amidases, which convert the amide to carboxylic acid, and also real nitrilases, which perform both reaction steps. An interesting nitrile hydratase was discovered which would selectively act on only one nitrile group in a dinitrile [2]. With this enzyme, adiponitrile can be converted to the monoamide and the remaining nitrile group hydrogenated in a traditional way, whereby caprolactam can be obtained in high yield. The process can be carried out in simple reactors at low temperature and pressure. This attractive process is still not commercialized. The main reason seems to be an overcapacity for *Nylon* production combined with the fact, that existing plants for caprolactam are already paid down whereby there is no economic initiative for closing these plants and build new ones even if the cost of the enzyme based plant is much lower. It is still a cost.

Parallel with this research, it was decided at *Novo* to look for enzyme processes which were suitable for forward integration, and where the enzyme step meant an absolute advantage.

Based on the vague idea that, if it was possible with an enzyme to couple sugar with lipid, the result might be an interesting new surfactant, a research project was initiated which, after several failures, in 1987 led to an interesting process. In this process, a hexose derivative is esterified with fatty acid using a lipase [3]. The process is interesting for two reasons. One is that by using a preferred enzyme, *e.g.* the lipase, component B, from *Candida antarctica*, the O–C(6)-monoester is formed almost exclusively. Thereby, a pure and well defined surfactant is obtained. This is normally not the case for industrial surfactants. The other interesting aspect is that the esterification takes place without solvents. This leads to appreciable savings in fixed costs, making it

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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.

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