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Application of Biotechnology at *Fluka*

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Abstract. Preparative tools based on the combination of chemistry and biotechnology have been used to make products of the required quality with respect to high-purity reagents for bioanalysis, or chiral products of high enantiomeric purity. Biotransformation with the development and production of the biocatalyst, stabilization and application of the biocatalyst are described as key elements to achieve these goals.

Introduction

To advance biotechnology into the next century, the exchange and application of existing knowledge of the past across borders, such as space-time borders, scientific, or language barriers, is a chance to be grasped. One particular area of the application of biotechnology at *Fluka* that has historically also benefitted from the intense exchange of ideas and knowledge over existing borders in chemistry and biotechnology is the area of biotransformations, although other application areas have this potential too. The existing application areas of biotechnology at *Fluka* are summarized in *Fig. 1*: the newest focus is on the analytical reagents in the field of bioanalysis and chemware, *i.e.*, tailor-made reagents which are adapted to their intended use together with analytical or preparative instruments. Since these instruments contain hard- and software, the required tailor-made reagents are, in analogy, called chemware-products.

In the area of biosynthesis, renewable biomass is used as a starting point for producing various chemical compounds. Another field where both the preparation of the necessary reagents and their application is performed in-house is biosepara-

tion, involving techniques such as aqueous two-phase extraction, membrane chromatography, and preparative electrophoresis.

In the following, I want to focus on the area of biotransformations, which, has attracted considerable attention [1–5].

2. Biotransformation

Although biocatalysts have been used in organic synthesis already early in the history of *Fluka*, the choice of a biotransformation, if chosen at all, was only for those products, where all other synthetic

methods worked poorly or failed. In the last 15 years, biocatalysts have been used more and more at *Fluka*, and today, there are about 100 such processes in routine production. The combination of biotechnology and organic chemistry has become a synthetic strategy already at the planning level.

In 1999, a new biotechnology laboratory with ten fermenters up to 300 l total volume for work with recombinant microorganisms has been established according to GMP.

The large-scale pharmaceutical applications are served
a) by a rapid development technology

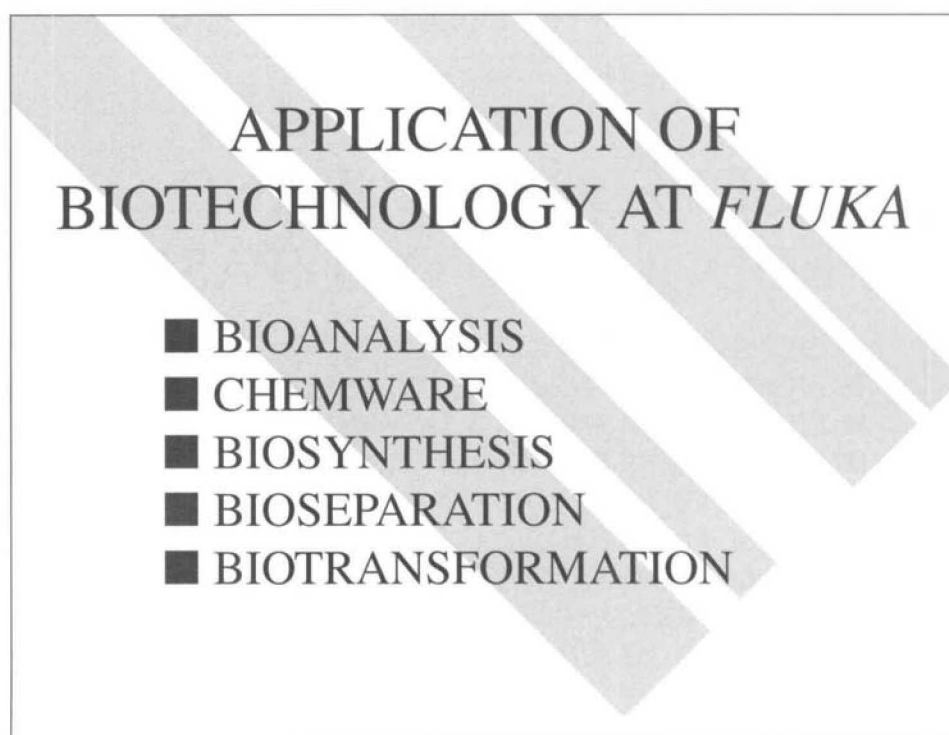


Fig. 1. Overview of biotechnology applications at *Fluka*

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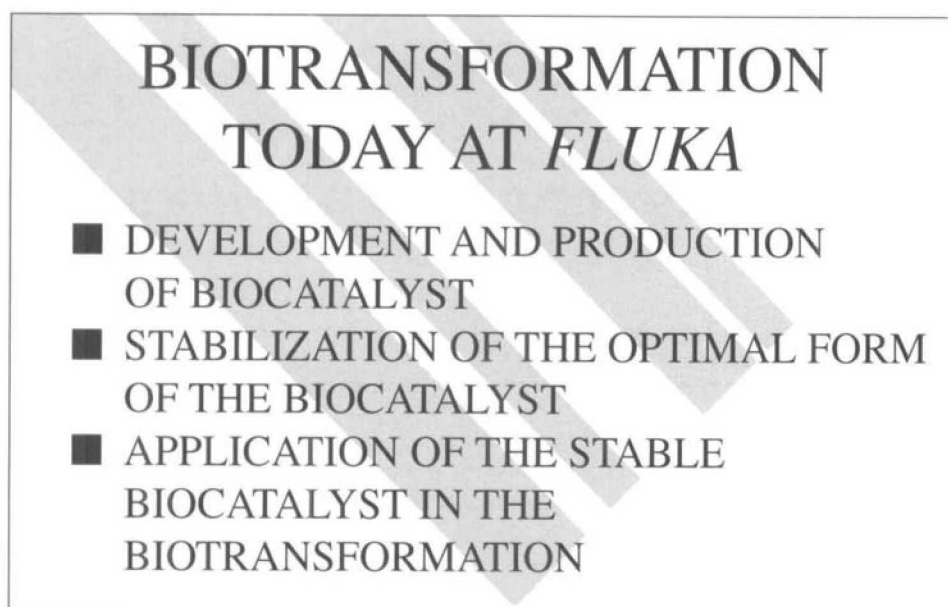


Fig. 2. Phases of a typical biotransformation project at Fluka

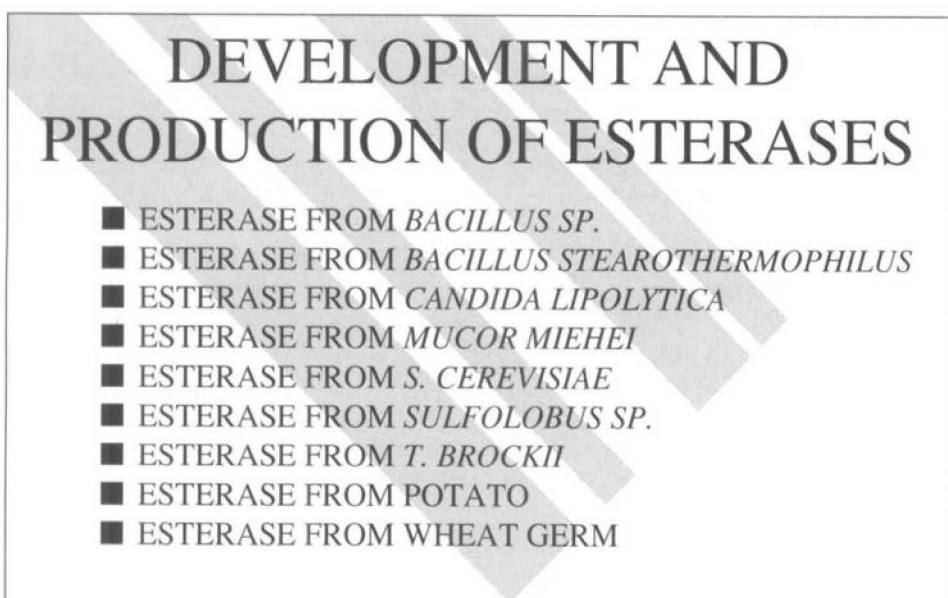


Fig. 3. Selection of developed microbial and plant esterases

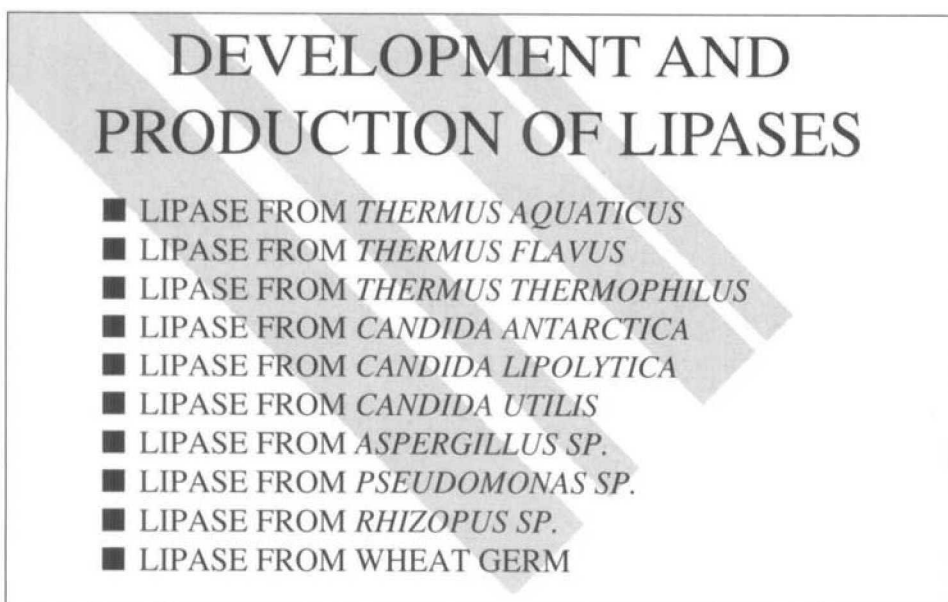


Fig. 4. Selection of developed microbial and plant lipases

going from discovery over the first samples to the first kilograms of biocatalyst and the corresponding biotransformation feasible with that particular biocatalyst,

- b) by using recombinant technology that allows to overexpress a certain biocatalyst and thereby getting higher volume yields, and
- c) by coupling biocatalyst production with applications, in which existing chemical reactors can be used.

The handling of a biotransformation project at *Fluka* today is illustrated in Fig. 2: the three areas biocatalyst production, stabilization, and application depend on each other and require good coordination to achieve a successful biotransformation.

2.1. Development and Production of the Biocatalyst

Since pig-liver esterase has been well established long ago as a broadly applicable biocatalyst, it was decided to extend the range of commercially available esterases to microbial and plant sources (Fig. 3). Sometimes, esterases and lipases can be produced by the same microorganism, depending on the fermentation and downstream processing conditions. A similar situation as with esterases is found for lipases, where pig-pancreas lipase has been broadly applied. A list of microbial lipases produced at *Fluka* is shown in Fig. 4.

Lipases and esterases have been the main contributors to the spread of enzymatic methods into our production, and, therefore, three kits have been assembled to make the unavoidable screening easier. New lipases and esterases from plants and microorganisms are being isolated, showing interesting substrate profiles [6–8].

An attractive entry to enantiomerically pure diols is the opening of easily accessible racemic epoxides by epoxide hydrolases, which do not require cofactors [9]. The preparation of the first commercially available epoxide hydrolase from *Rhodococcus rhodochrous* has been recently completed [10].

A more complex biotransformation involving cofactors is the *Baeyer-Villiger* oxidation, which was discovered 100 years ago [11] but which is chemically still not feasible in an enantiospecific way [12]. In Fig. 5, seven described microbial monooxygenases have been prepared for use in enzymatic *Baeyer-Villiger* oxidations [13][14].

2.2. Stabilization of the Biocatalyst

Whenever possible, the biocatalyst is lyophilized, not only to keep it stable over longer periods of time, but also for greater

flexibility in the application concerning reaction parameters like solvent and educt/product solubilities. If required, the biocatalyst can also be stabilized by immobilization, which is achieved at *Fluka* by two different techniques, which are shown in *Fig. 6*: the biocatalyst is either covalently coupled to the epoxy-group of a polymer like *Eupergit C* [15], or the biocatalyst is noncovalently entrapped with the use of alkoxysilanes in aqueous buffer solutions, whereby hydrolysis leads to a sol-gel transition, and robust enzyme silica gel can be produced [16].

2.3. Application of the Biocatalyst

The ways in which biocatalysts have been applied are continuously expanding, and the application itself can become the target of biocatalyst development (*Fig. 7*). Four groups of compounds are currently being produced with biocatalysts, as can be seen in *Fig. 8*. The final application of the biocatalyst shows the strengths and weaknesses of the system: either, successful biotransformation is achieved, or not achieved. If the production process shows that the biocatalyst is the problem, a biocatalyst with improved performance [17][18] for the same biotransformation has to be found, or other synthetic routes should be envisaged.

DEVELOPMENT AND PRODUCTION OF MONOOXYGENASES

- CYCLOHEXANONE MONOOXYGENASE FROM *ACINETOBACTER SP.*
- CYCLOHEXANONE MONOOXYGENASE FROM *NOCARDIA GLOBERULA*
- CYCLOHEXANONE MONOOXYGENASE FROM *XANTHOBACTER SP.*
- CYCLOPENTANONE MONOOXYGENASE FROM *PSEUDOMONAS SP.*
- PROGESTERONE MONOOXYGENASE FROM *CYLINDROCAPRON SP.*
- TRIDECANONE MONOOXYGENASE FROM *PSEUDOMONAS PUTIDA*

Fig. 5. Selection of developed microbial monooxygenases

STABILIZATION OF BIOCATALYST BY IMMOBILIZATION

Core Technology	Products in the Bottle	Perspective
<p>1) Covalent Coupling of the Enzyme to the Epoxy Group of Polymer</p>	<p>Acylase, Catalase, Esterase, Urease Lipase, Papain Penicillin Amidase</p>	<p>Scale-Up Easy Application Easy New Developments Easy</p>
<p>2) Entrapment of Enzyme by Sol-Gel-Process with Silanes</p>	<p>Lipase, Esterase</p>	<p>Scale-Up Possible Application Easy New Immobilizations Empirical</p>

Fig. 6. Immobilization technologies used for biocatalysts at Fluka

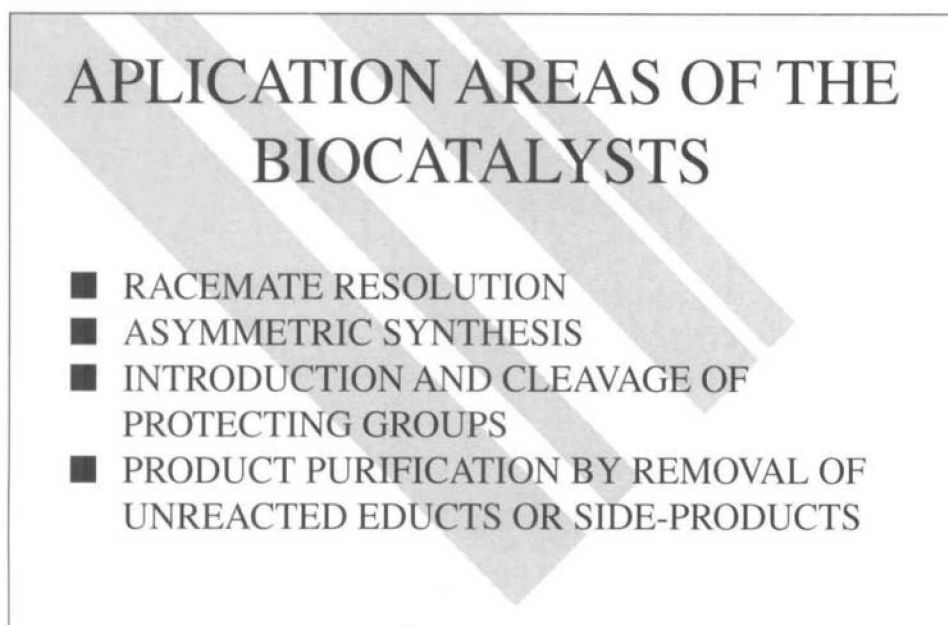


Fig. 7. Current application areas for biocatalysts in organic synthesis at Fluka

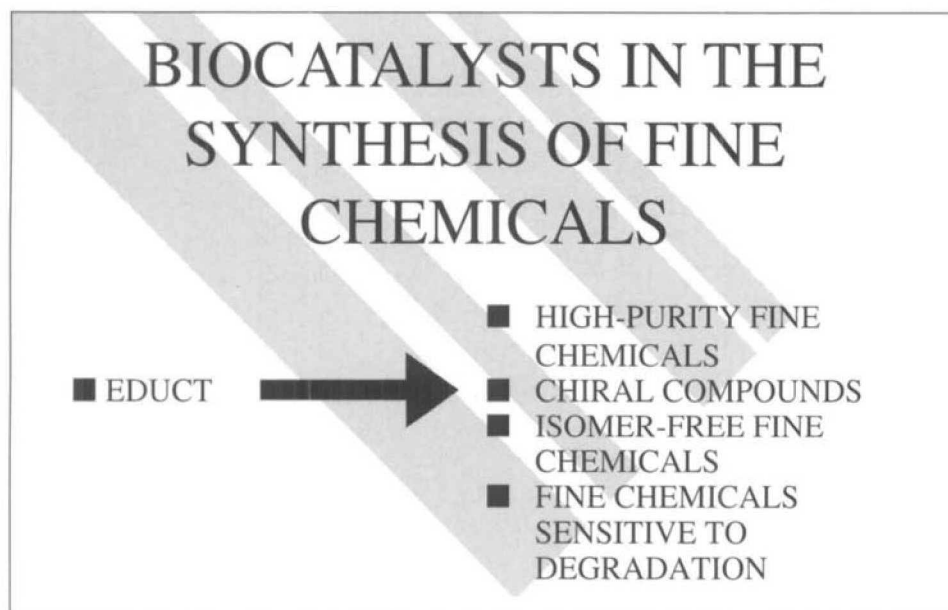


Fig. 8. Classes of fine chemicals manufactured at Fluka using biocatalysts

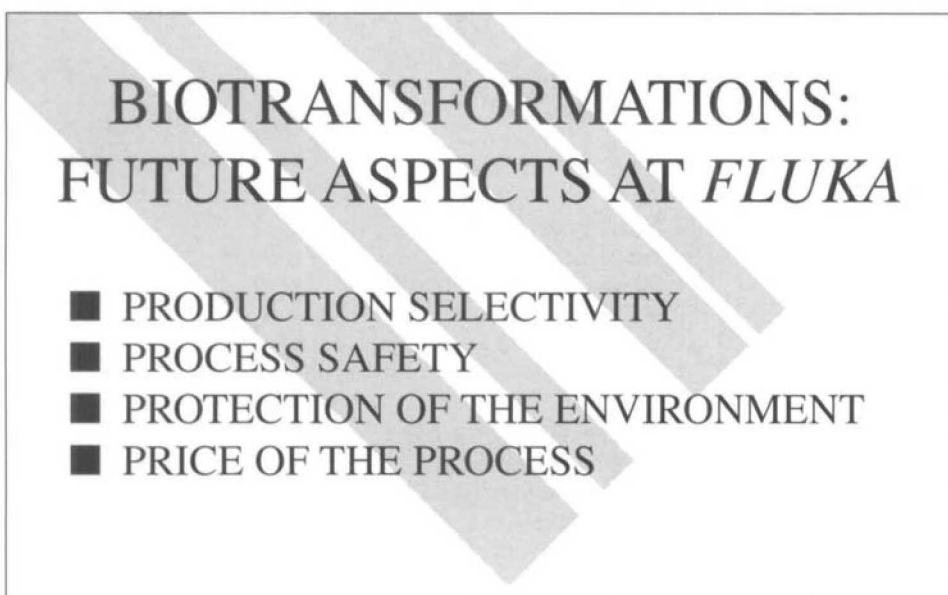


Fig. 9. Biocatalyst-application criteria of potential future interest

3. Outlook

Aspects like selectivity of the production process, protection of the environment, process safety, and, last but not least, the price of the process might well shape the future face of manufacturing (Fig. 9). Since the molecules to be synthesized in the future will be more complex, I am convinced that biocatalysis (also including whole-cell biocatalysis) will play an even stronger role in the next century.

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