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Biotechnological production of l-tryptophane as a pharmaceutical ingredient : optimization of the process by immobilization and use of bioanalytical systems

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Biotechnological Production of L-Tryptophane as a pharmaceutical ingredient: optimization of the process by immobilization and use of bioanalytical systems

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1 Introduction

L-tryptophan is an essential amino acid, which occurs naturally in all organisms. In humans, it has a sedative and antidepressive effect in higher doses although it is neither addictive nor physiologically harmful. Due to this unique combination of features, there was a fast developing market for this amino acid as a soft sleeping drug in the 1980s. A further application of L-tryptophan is as ingredient in medical infusion for pre- and postoperative, parenteral nutrition.

L-tryptophan has been produced at the AMINO GmbH for 12 years using a biotechnological method as displayed in Figure 1 (Wagner et al., 1980, Faurie and Fries, 1999).

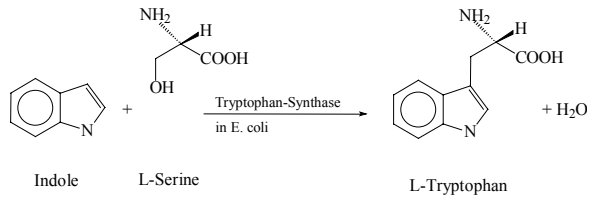

Figure 1:
Enzymatic synthesis of tryptophan

A strain of *E. coli* overproducing a highly active tryptophan synthase is fermented in a 3 m^3 scale. After separation of the biomass, the cells are mixed with a solution containing serine and indole. Serine is produced by chromatographic extraction from sugar beet molasses. Indole as a reaction partner with low solubility is added during the transformation process. The optimal dosage profile ensures a high space-time-yield and a low byproduct concentration. Cells are permeabilized in situ by indole, so that the reaction with the intracellular tryptophan synthase can take place. If indole is supplied in too high concentrations, the enzyme is inhibited whereas too low concentrations lead to a suboptimal space-time-yield and higher amounts of undesired by-products.

After the biotransformation, most of the biomass can be recovered by separation and is mixed with fresh biomass to be used again for further transformations. After several biotransformation cycles, biomass has to be discarded due to the decrease in enzymatic activity.

2 Process optimization by immobilization

The process can be optimized by increasing the stability of cells and enzyme activity. After fermentation, cells can be either cross-linked or entrapped in suitable materials. Combination of both methods can be efficient. The entrapment of the cell was achieved by using polyvinylalcohol (PVAL), the method was recently described (Jekel et al., 1998). This hydrogel provides immobilized biocatalysts with ideal features.

3 Entrapment in LentiKats®

E. coli cells are mixed with a highly viscous suspension of 1 part of polyvinylalcohol (supplied by Fa. geniaLab, Braunschweig) and 4 parts of water. By dropping or stamping, lentile shaped little tiles are formed (approx. 200 to 400 µm thick, diameter of 3 to 5 mm). At defined humidity and temperature, a hydrogel is formed by developing hydrogen bonds. These LentiKats® (Figure 2) are very resistant to mechanical and chemical stress. They can be used up to 50°C. Due to the unique shape and physical properties, they show very good diffusion features, so that transport processes are only limiting in extreme situations.

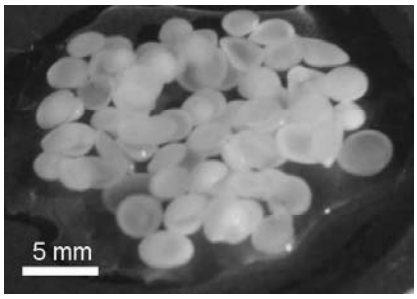

Figure 2:
Some LentiKats® shown by light microscopy

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4 Comparison of LentiKats® and free cells

20 transformations were carried out with free cells or LentiKats® under the same experimental conditions. In both cases the cells or biocatalyst were reused each time. The result of those biotransformations are shown in Figure 3.

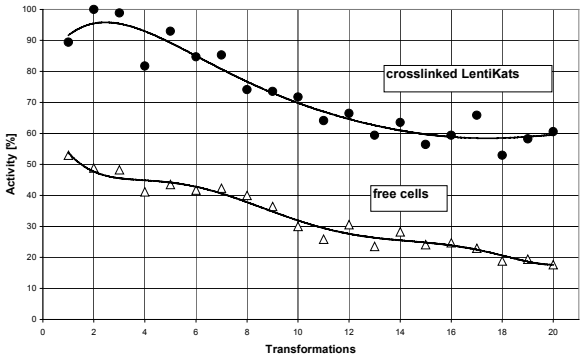

Figure 3:
Comparison of the activity of tryptophan synthase of free cells and LentiKats®

The entrapment of the cells into hydrogel results in a higher activity, decreasing in much lower degree than for free cells. Thus a higher process stability can be reached by this method.

5 Impact of cross-linking

An increased activity and higher stability of the separated cells can be achieved as well by cross-linking with glutaraldehyde. After fermentation, the separated cells were mixed with 10 mM glutardialdehyde. Activities of different treated cells were compared (Figure 4). Results are shown for a) not cross-linked, free cells; b) cross-linked, free cells; c) not cross-linked, immobilized cells; and d) cross-linked, immobilized cells. A combination of cross-linking and immobilization is most effective, stability of the biocatalysts can be increased by more than 100%.

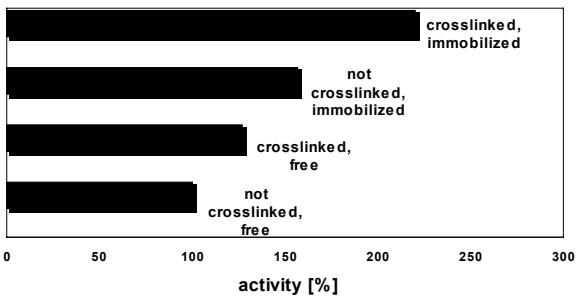

Figure 4:
Activity of different treated biocatalysts (mean of 12 transformations)

6 Process optimization by use of 2-D-process fluorimetry

During several biotransformation cycles suitable fluorescence spectra were recorded. This allowed an in-time determination of the concentrations of tryptophan and indole. The recorded data were used for the construction of a mathematical algorithm for the prediction of the process parameters. With application of this model an optimized dosage of indole can be achieved (Ulber et al., 2001).

7 Conclusions

Costs for downstream processing can be minimized by immobilization of the used biocatalysts. The separation step of the biomass after transformation can be achieved by simple sedimentation, just by turning off the stirrer. After sedimentation of the biocatalyst the product solution can be pumped out of the vessel. Time and equipment (separator) can be saved.

The costs for the biocatalysts are also decreased by minimizing the loss of bacterial cells during separation. Furthermore, the immobilized biocatalysts possess a higher long term activity which results in a lower consumption of cells. Assuming the costs for hydrogel and entrapment process to be 1-5 EUR per kg, costs for the biocatalysts can be reduced about 30 up to 80 %.

Free inactivated biomass can be released to waste water plants after use. LentiKats® are liquefied by heating to 70°C. For many biotechnological processes the step is obligatory for the inactivation of the biomass, so that additional costs will not occur. For keeping the melted polymer in liquid condition up to temperatures of 4°C, the suspension has to be diluted with one volume of water or aqueous process effluents.

In biological waste water plants, degradation of the hydrogel up to > 90 % is possible (Schonberger et al., 1997). Basic requirements are adapted microorganisms and temperatures above 12°C. The adaption phase of the microorganisms takes 3 to 6 weeks.

By application of 2-D-process fluorimetry based in-time analytics a higher product concentration can be obtained in shorter times. This leads to a significant reduction in consumption of the educts indole and serine. Additionally the power demand for downstream processing and waste water load are decreased.

8 Outlook

Whereas reaction control by use of 2-D-spectro fluorimetry could be tested on a production scale, all immobilization experiments have thus far been carried out on a laboratory scale. Prior to transfer to the

production scale, companies producing pharmaceutical ingredients must fulfil high quality GMP standards. These include that neither the use of polymers nor of glutaraldehyde affects the final quality of the pharmaceutical products.

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