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natural renewable raw materials. 87% of the raw materials used in the fermentation is glucose as opposed to 23% glucose used in the chemical synthesis. This reduces the use of organic solvents and other chemical substances and yields 36% less air emissions and wastes. Furthermore, energy consumption can be reduced by 25%.

Conclusion

Development of a successful commercial process using 'modern' biotechnological approaches requires not only the construction of a high-performing strain and optimization of a fermentation process, but also major activities related to product quality, safety, and acceptance. The use of recombinant technology for strain improvement makes premarket approval mandatory in many countries, which is not necessarily the case for 'classical' mutation-selection approaches. The extensive reviews done by the various national authorities during the evaluation process ensure optimal product safety. Thus, despite its image with parts of the general public, use of recombinant DNA technology adds to the safety of the products produced.

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Bioprocess Technologies Depending on the Molecular Structure of Pharmaceutical Products

Berthold G.D. Bödeker*

The source material for biopharmaceuticals varies considerably comprising plant or animal organs, human plasma, microbial or mammalian cell fermentation, transgenic animals and others. Products include secondary metabolites as well as natural and recombinant peptides and proteins. Each of these products depending on the source material requires its own bioprocess technologies to result in a product of high quality and safety for human use. Three examples of biopharmaceuticals are described covering the range from a secondary metabolite from large-scale actinomycete fermentation to a protein obtained from organ extraction to a recombinant protein from continuous fermentation of genetically engineered mammalian cells.

**Correspondence*: Dr. B.G.D. Bödeker
Bayer AG, Pharma-Biotechnology
Friedrich-Ebert-Strasse 217
D–42096 Wuppertal

Acarbose – a New Antidiabetic Drug from Actinoplanes

The pseudo tetrasaccharide Acarbose consisting of an acarviosin and a maltose unit is a competitive inhibitor of α-glycosidases. Clinically it is used for diabetes-type-I and -II therapy representing a novel principle in diabetes treatment by delaying the digestion of polysaccharides.

Acarbose is a secondary metabolite from *Actinoplanes spec.* It is commercially manufactured in large-scale fermentation followed by a multistep purification process. In order to achieve an economical process an extensive amount of process development had to be performed, since the product – as most secondary metabolites – was originally produced in very small quantities by the cells. The main focus in process development was therefore a strain improvement program by stepwise selection using various mutagens and protoplast fusions. Indeed, it was possible over a long period of time to increase productivity of the *Actinoplanes* cells by orders of magnitude. A second focus of process development was the fermentation process. Here, it was important by fine tuning of the cultivation parameters to maximize product yields and minimize concentration of similar by-products which are difficult to separate from Acarbose during down stream processing.

After more than a decade of intense development it has been possible to achieve a commercially viable large-scale process to produce Acarbose in the hundreds of tons scale, whereas particularly strain improvement and accompanying fermentation and purification development are still ongoing to succeed in an even more economical process.

Aprotinin – a Protease Inhibitor from Bovine Lungs

Trasylol®, which is the trade name for the protease inhibitor aprotinin from bovine lungs, is used in the clinical indication of open heart surgery to minimize blood loss. The protein with a molecular weight of 6512 represents a classical extraction product manufactured by solvent extraction of bovine lungs followed by

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purification mainly through several column chromatography steps.

In order to use animal source pharmaceuticals in humans, safety considerations, particulary viral safety, are of high importance in addition to purity and efficacy. The major safety concern regarding bovine tissues is Bovine Spongiform Encephalopathy (BSE), a degenerative disease of the central nervous system affecting cattle. In order to demonstrate the BSE reducing capacity of the *Trasylol®* manufacturing process, a validation study was performed using the four process steps: alcohol extraction, two column chromatography steps and a selective ultrafiltration through a membrane with a molecular weight cut off at 10000 [1].

Lung material was spiked with high infectious titers of the rodent adapted scrapie ME 7 as model for BSE and processed through the scaled down version of the manufacturing steps. The samples were then tested in C57BL mice carrying the Sinc gene. An 18 log reduction in titer of infectious agent was observed. These results in addition to all other quality control measures and the fact that the bovine lungs are only obtained from countries which are considered to be BSE-free indicate an extremely high BSE safety factor in *Trasylol®* production.

Recombinant Factor VIII – a Glycoprotein from Perfusion Cultures of Mammalian Cells

Coagulation Factor VIII deficency is an inherited disease resulting in uncontrolled bleeding episodes in Hemophilia A patients. Treatment of Hemophilia A patients is done by applying external Factor VIII formerly obtained from human plasma and now available as recombinant Factor VIII (rFVIII). *Kogenate®* which is the tradename for rFVIII from *Bayer* represents the first worldwide licensed recombinant glycoprotein manufactured from continuous perfusion culture of genetically engineered mammalian cells. In addition, rFVIII is the first worldwide approved recombinant protein for chronic life-time treatment.

For *Kogenate®* production [2] baby-hamster-kidney 21 cells transfected with the human Factor VIII gene are used. These cells are cultured in a specifically designed serum-free medium that allows high yield expression of this complex, highly glycosilated protein. Large-scale cultivation is performed in deep-tank fermenters using a continuous, high cell density cultivation process. The suspension cultures are operated in a perfusion mode for up to six months under steady state conditions, allowing a high degree of culture control. High cell density is achieved by in house developed cell retention systems yielding up to 30-fold increased densities compared to batch or fed batch cultures. Thus, a 500 l perfusion culture produces as much rFVIII as a 15000 l batch fermenter.

The rFVIII containing harvests from fermentation are processed batch-wise through a multistep purification system including ion exchange, gelfiltration and affinity chromatography. The key step is immunadsorption chromatography using monoclonal antibodies against Factor VIII.

The antibodies are also produced in a continuous perfusion process from hybridoma cells, purified and coupled to a glass beads matrix. The rFVIII purification process is very powerful in removing host cell and other impurities from the product. The final impurity levels are in the picogram range per clinical dose for DNA and nanogram range per dose for protein impurities.

In addition the purification process has the capability to remove viruses which is achieved by a combination of virus inactivation and virus clearance of the chromatography steps. A validation study showed that the overall titer reduction was 6–12 logs depending on the model virus.

The worldwide approval of *Kogenate®* indicate that the production concept of continuous perfusion fermentation coupled with batchwise purification is an efficient alternative to batch cultures for the production of pharmaceutical proteins from mammalian cells.

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Industrial Bioprocesses for the Production of Substituted Aromatic Heterocycles

Rainer Glöckler* and **Jean-Paul Roduit**

1. Product Range

The chemical oxidation of alkylated pyridines, pyrazines, and pyridine- and pyrazinecarboxylic acids and the ring hydroxylation of *N*-heteroaromatic carboxylic acids often leads to formation of by-products caused by non-specificity of the chemical reaction. We have developed several biotransformation reactions for the production of specifically functionalized aromatic *N*-heterocycles. For example, the regioselective enzymatic oxidation of methyl groups has been extensively studied.

1.1. *Enzymatic Oxidation of Methyl Groups*

Pseudomonas putida ATCC 33015 grown on xylene as sole carbon and energy source, oxidizes many methylated heteroaromatic five- and six-membered rings to the corresponding monocarboxylic acids (*Scheme 1*). The oxidation of 2,5-dimethylpyrazine to 5-methylpyrazine-2-carboxylic acid (MPCA) was studied in more detail resulting in a 15-m^3-scale production process reaching concentrations of 24 g MPCA/l with an analytical yield of >95% [1].

**Correspondence*: R. Glöckler
Biotechnology Department
Lonza AG
CH-3930 Visp