Chimia 53 (1999) 562–565

ISSN 0009–4293

Production with Bacterial and Mammalian Cells – Some Experiences

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Abstract. Bridging the gap between academia and industry, between basic and applied research, was one of the targets of the 1st Czech-Swiss Symposium on Advanced Biotechnology held in Prague, September 4–7, 1999. This presentation identifies some selected areas in industrial biotechnology, where we see potential for improvement. Our proposals are based on our experience with biotechnological processes, such as immobilised enzyme processes, whole cell fermentation processes, and mammalian cell culture, with products ranging from optically pure chemicals to monoclonal antibodies and proteins, and from our alliance with *Genzyme Transgenics* for the isolation and purification of selected proteins from transgenic animals. Since strain and process design for fermentation are key issues, and downstream processing (DSP) is dependent on steps upstream, mainly fermentation, we will focus our discussion on the fermentation step.

1. Introduction

The advantage of biotechnology lies in the correct assembly and folding of large multichain proteins, including post-translational modifications, notably glycosylation, with mammalian cells and in the chemo-, enantio- and regioselectivity of the enzymes for the production of complex fine chemicals.

Table 1 summarises all the technologies available today, which can be used for production of proteins and/or fine chemicals. The production with mammalian cells, and especially with bacteria and yeasts, are established tools, but the production with transgenic animals and plants is also rapidly progressing, and transgenic animals already now represent an additional choice for production. Although plants are a rich source of biologically active fine chemicals, plant cells in suspension culture are not yet routinely used for large-scale production. There have been a few exceptions in the past, the most prominent one being the synthesis of shikonin developed by *Mitsui Petrochemical Company* in Japan.

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The production of therapeutic proteins using mammalian cells *e.g.*, CHO (chinese hamster ovary), BHK cells (baby hamster kidney), mouse cell lines (*e.g.*, C127, NSO), or microbial cells has been reported elsewhere [1][2], as well as the production of, *e.g.*, functionalised aromatic and aliphatic *N*-heterocycles and other fine chemicals frequently found in biologically active man-made pharmaceuticals [3][4].

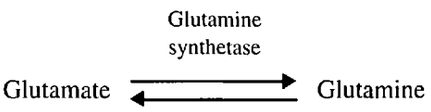
Irrespective of the organism used in suspension culture processes for biotechnological manufacturing, there are numerous common problems, but also typical differences, which will be discussed below. The strain development and process design of fermentation are the key issues for a commercially viable bioprocess, and this paper consequently focuses on these aspects.

2. The Production Strain

Table 2 summarises our experience concerning the different factors influencing manufacturing costs with mammalian and bacterial cell cultures. Generally, the strain and its growth characteristics define medium composition, cycle times, and final product concentrations. Consequently, steps and yields of downstream processing (DSP) and volumetric sterile productivity (the two key cost drivers) are directly related to the strain.

The ideal production strain is genetically stable, has a high specific (q_p) and volumetric productivity (Qp), forms no byproducts, and uses a well-defined medium resulting in a DSP with limited steps and, consequently, low losses in yield during DSP. In reality, the DSP is often still the most costly manufacturing step, because of insufficient strain productivity and the use of complex media formulations.

A key issue in achieving a high specific production rate for a recombinant protein or a fine chemical is the choice of a highly efficient gene-expression system [5]. In the case of mammalian cells, *Bebbington et al.* [6] described a new selection system, in which the glutamine synthetase (GS) was incorporated into expression vectors along with the product gene(s). The GS gene confers glutamine-independence on the cell line into which the vector is transfected, allowing simple selection of transfectants by their ability to grow in glutamine-free medium [7].



The use of strong promotor sequences ensures efficient product-gene expression.

Using, for example, mouse myeloma cells together with the GS system, product titers of 1 g of monoclonal antibody per

litre have been observed in batch culture [1][7].

There is an increasing interest in the use of genetic engineering technology and variant selections to improve the metabolic characteristics of mammalian and microbial production strains. Examples include the isolation of cholesterol-independent clones of mouse cells [7] and the introduction of anti-apoptosis genes [8], or the alteration of glycosylation pathways.

The genome of over 20 different bacteria and of yeast have been sequenced in totality, and this number of known genomes increases. This knowledge will soon have impact on the industry, especially with the identification of the as yet unknown functions of these genes. But since most microorganisms and their enzymatic potential are still unknown, high-throughput screening is another important tool to search for new enzymatic ways for the production of fine chemicals. Unfortunately, the only detailed documentation available is on hydrolytic enzymes, notably proteases, esterases, and lipases [4]. Of the six classes of enzymes, five remain to be investigated. This is indeed an important research area, and, therefore, it is worrying to see that the annual number of publications on enzyme research is decreasing.

3. The Medium

The main purpose of DSP is to remove unwanted media components and byproducts produced by the production organism. It is known that carbon overflow in bacteria limits cell production, reduces protein production, and compromises process stability [9]. Even phage titers in *E. coli* can be dependent on the medium composition [10]. Statistical experimental design combined with pulse and shift techniques in chemostats are useful tools for medium design and strain selection by continuous selective pressure [7][11]. In combination with statistical tools, modelling techniques complementing purely empirical procedures accelerate and simplify process development in general and not only for medium design [12]. Fed-batch technology, extensively used in microbial fermentations, is now also used in mammalian cell culture to prolong the production time of batches [13][14] and to improve the ratio between product and waste. The development of defined media for microbial and for mammalian cells is a difficult but important step toward better yields in DSP. Defined media also provide better process definition, which is a benefit in process optimisation. The avoidance of animal-derived raw materials, such as proteins, also excludes the possibility of introducing unwanted microbial agents into the process.

Table 1. *Systems that Can Be Potentially Used for the Biotechnological Production of Fine Chemicals and Proteins.* – = difficult; +++ = advantageous.

	Fine chemicals	Proteins & monoclonal antibodies	Glyco-sylation	Cost advantage	Product safety	Sterile operation
Prokaryotes	yes	+	–	+++	++	+
Yeasts	yes	+	+	+++	++	+
Plant cell culture	yes	+	++	+	+++	–
Insect cells	no	+	++	+	+	–
Mammalian cells	no	+++	+++	–	+	–
Transgenic plants	yes	+	++	++	++	+++
Transgenic animals	no	+++	.+++	++	–/+	+++

Table 2. *Effect of Strain, Process and Plant on the Overall Process Outcome*

	Process flexibility	Effect on Q_p	Effect on sterility	Effect on cost
Strain	***Key***	***Key***	Cycle time	***Key***
Process				
Medium	Limited	Limited	Can be important	Medium
Parameters	Limited	Very limited	Usually limited	Small
Plant				
Fermentation	Very limited	Moderate	***Key***	I & D
DSP	Yes	Small	Product-dependent	I & D & Y

Q_p = volumetric productivity. I & D = Interests and depreciation. Y = yield.

4. Parameters and Their Control

Two parameters differ by an order of magnitude between bacterial and mammalian cells: growth rates (0,1–1/h for bacterial cells *vs.* 0.01–0.05/h for mammalian cells) and oxygen-uptake rates (OTR>5 mmol O_2/g/h for bacterial cells *vs.* OTR= 0,05 mmol O_2/g/h for mammalian cells) [15][16].

The importance of growth rates for the production of recombinant therapeutic proteins with prokaryotes has been well known for many years [17][18]. Prokaryotes and yeasts rarely produce proteins at maximum growth rates (μ_{max}), and similar observations have been made with higher eukaryotes as well. Growth rate and control are important parameters.

However, reliable real-time on-line measurement of these factors is still difficult and relies mostly on direct optical or indirect methods.

5. Process and Plant Design and Engineering

Microbial, plant, and mammalian cells show differences other than growth rates and OTR. Mammalian cells are far more shear-sensitive than bacterial cells, although the degree of sensitivity is often overestimated. This shear-sensitive nature, combined with low growth rates and the lower oxygen consumption, has a major impact on the type and design of reactor used. The reactor design for mammalian

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cell culture and the reasons for cell damage occurring during suspension culture have been discussed elsewhere [19][20]. Shear-sensitivity of mammalian cell cultures, rarely encountered with prokaryotes and yeasts, is proposed to be due to

- direct collision of cells with baffles
- pressure difference between front and back side of baffle
- micro eddies smaller than the cells
- degree of turbulence
- air-bubble/cell interactions, predominantly bubble bursting

Shear-damage can usually be avoided by use of the appropriate medium and sophisticated reactor design. Medium supplements can either improve the robustness of cells (*e.g.*, serum or synthetic polymers, surfactants) or change the viscosity of the medium (carboxymethylcellulose) and, consequently, the rheological characteristics of the medium or the influence of interactions between cells, liquid and bubbles. Improved design for agitation and/or aeration, or even alternative fermentation types should reduce shear stress while maintaining good mass-transfer characteristics. For example, the torus reactor shown in *Fig. 1* was demonstrated to have a low specific power input and lower hydrostatic resistance for aeration [21]. This low-shear agitation system was suggested for tests with animal cell culture [20]. Comparative studies of different bioreactor designs, as available for microbial systems [22], are unfortunately lacking for mammalian cell culture. Such an investigation is very important for mammalian cells, because different systems are in use (airlift, stirred tank, perfusion system, adherent, *etc.*). It is, however, interesting that for such a homogeneous group of products (proteins), so many different fermentor types are being used. We speculate that the reason for this is the lack of comparative fermentation-performance studies.

In contrast to mammalian cells, microbial cells can almost always be cultivated successfully in a continuously stirred tank with *Rushton*-type impellers and an aver-

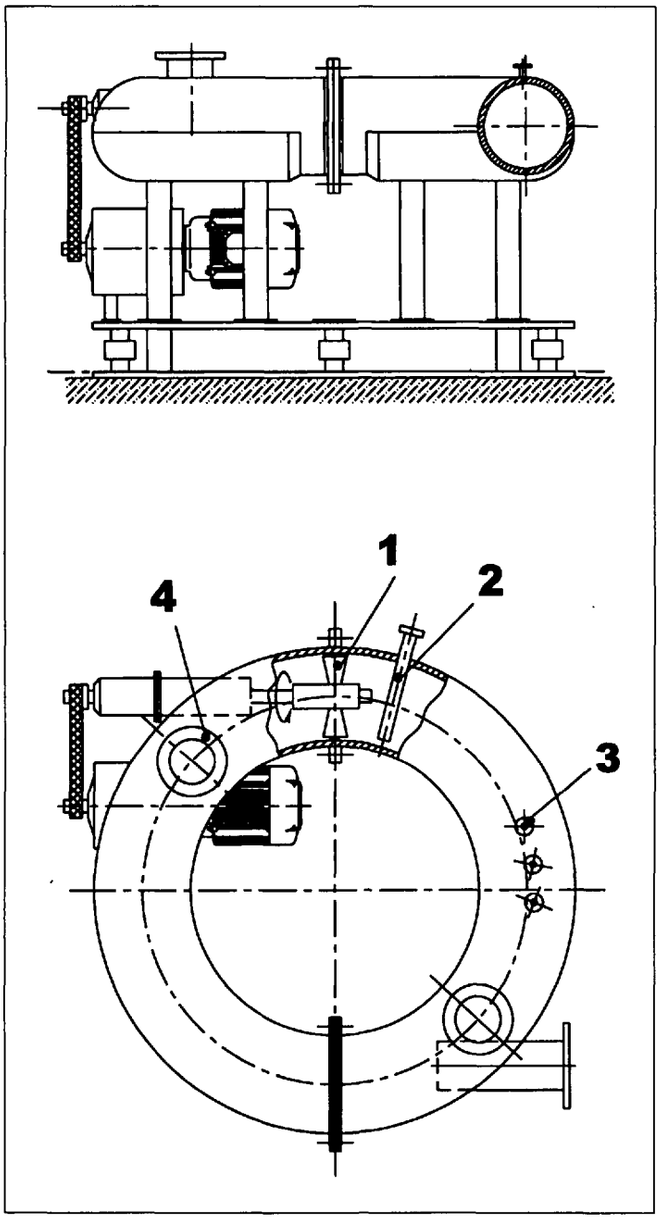

Fig. 1. *Cross-section and overview of a torus bioreactor.* 1. Marine impeller moving the fermentation medium into circular motion. 2. Air sparger downstream from impeller. 3. Ports for sensors. 4. Handhole.

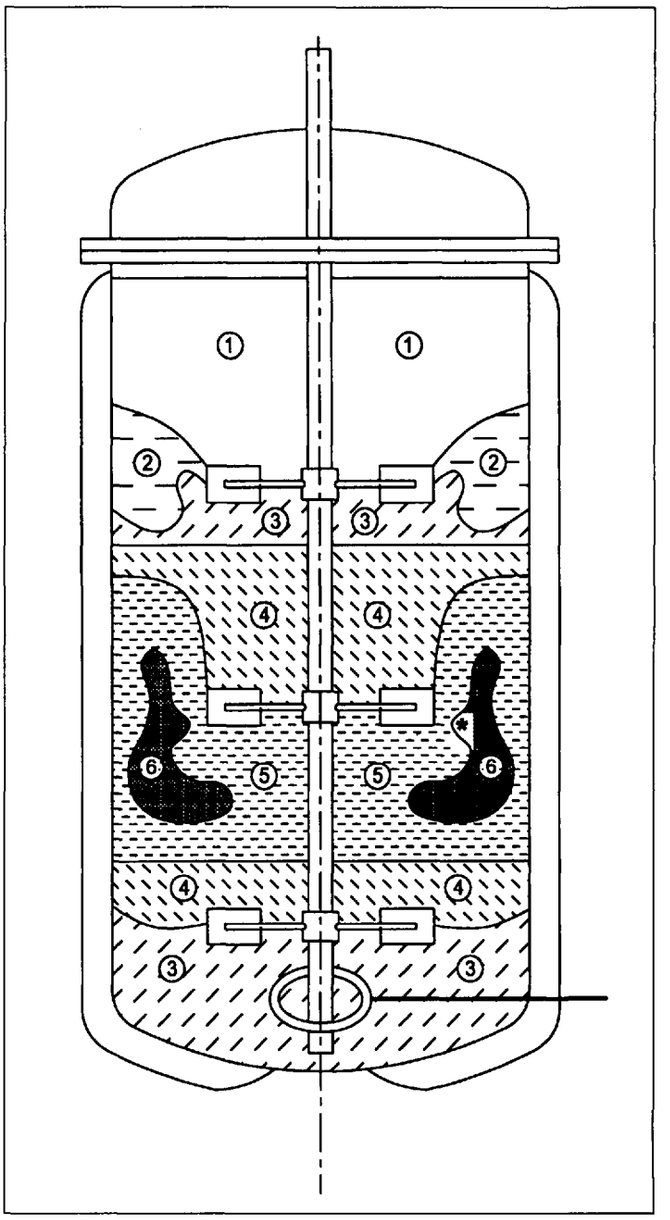

Fig. 2. *Concentration distribution in a 15-m³ reactor of an educt fed into the reactor at one point * (feed point).* 1: 99.2%; 2: 99.3%; 3: 99.5%; 4: 99,6%; 5: 99.7%; 6: 99.9%; feed point *= 100%.

age height-to-diameter ratio of 3:1. This design can handle low-viscous and high-viscous broth and delivers the high mass and heat transfer usually requested by microbial cells. Manufacturers need to have a great deal of detailed knowledge on what is going on in the reactor. *Fig. 2* shows the small concentration gradients which can be reached in a 15-m^3 fed-batch fermentation if the feed-pipe and the stirrers are properly designed (*Hoek*s, unpublished results [23]). Also, sometimes, a simple design change can have a big effect. One example is the increase of the production volume of a reactor by foam prevention and foam reduction [24].

Product inhibition by protein is rarely observed in microbial or mammalian cell culture. However, product inhibition is a specific phenomenon often observed when producing fine chemicals with microbial cells. We tried to solve this problem, together with the Swiss Federal Institute of Technology in Lausanne [25], by continuous removal of the inhibiting product by ISPR (*in situ* product recovery) during the actual fermentation. While we have found practical solutions at the 20-l scale, we are still facing difficulties with larger reactors. Scaling-up and long-term reliability are problems also found with continuous fermentation and production, although volumetric productivities are usually much higher [26].

5. Sterility

Sterile technology and operation is a *conditio sine qua non*. Bacterial contaminations, phages and mycoplasmas must be kept at bay. Even with today's state-of-the-art technology, there are sterility challenges left. Examples of problematic sterile operations are

a) Addition of water-insoluble substrates
b) Long duration of fermentations
c) Sterile feed of several nutrients and pH control.

The sterile design of a microbial reactor does not necessarily differ from a mammalian fermentor, since the cycle times of microbial fermentations or semicontinuous fill and draw operation can reach or even exceed durations observed in mammalian cell culture. However, with state-of-the-art equipment and observing the following basic rules, contamination levels close to 0% are possible:

- Continuous training of the operators
- Proactive maintenance
- Reduce all internal parts (cooling coils, bolts, nuts) of the fermentor as much as possible
- Do not use flanges, but weld
- Use Pt-100 at all critical points of the fermentor
- Make sure all air is replaced with saturated steam (can be difficult in very large fermentors)
- Prevent foaming when breaking the vacuum
- Observe condensate collectors
- Assure continuous 2% slope in all transfer lines

Received: September 31, 1999

[1] J.R. Birch, J. Bonnerjea, S. Flatman, S. Vranch, in 'Monoclonal Antibodies: Principles and Applications', Wiley-Liss Inc., 1995, p. 231.
[2] A.S. Lubiniecki, A.S. Vargo (Eds.), 'Regulatory Practice for Biopharmaceutical Production', 1994, p. 1.
[3] M. Petersen and A. Kiener, *Green Chemistry* **1999**, *1*, 99.
[4] H.-P. Meyer, A. Kiener, R. Imwinkelried, N. Shaw, *Chimia* **1997**, *51*, 287.
[5] H. Hauser, R. Wagner (Eds.), 'Mammalian Cell Biotechnology in Protein Production', W. de Gruyter Publishers, 1997.
[6] C.R. Bebbington, G. Renner, S. Thomson, D. King, D. Abrams, G.T. Yarranton, *Bio/Technology* **1992**, *8*, 662.
[7] J.R. Birch, R.C. Boraston, H. Metcalfe, M.E. Brown, C.R. Bebbington, R.P. Field, *Cytotechnology* **1996**, *15*, 11.
[8] H. Bierau, A. Perani, M. Al-Rubeai, A.N. Emery, *J. Biotechnol.* **1998**, *62*, 195.
[9] H.-P. Meyer, H.-J. Kuhn, S.W. Brown, A. Fiechter, 'Proceedings 3rd Eur. Congr. Biotechnol', Munich, 1984, Vol. 1, p. 499.
[10] D.W. Clarke, H.-P. Meyer, C. Leist, A. Fiechter, *J. Biotechnol.* **1986**, *3*, 271.
[11] H.-J. Kuhn, U. Friederich, A. Fiechter, *Eur. J. Appl. Microbiol. Biotechnol.* **1997**, *6*, 341.
[12] M. Rohner, H.-P. Meyer, *Bioprocess Engineering* **1995**, *13*, 69.
[13] J.R. Birch, 'Bioprocess Technology: Fermentations, Biocatalysis and Bioseparations', John Wiley and Sons Inc., 1999, p. 2509.
[14] T.A. Bibila, D.K. Robinson, *Biotechnol. Prog.* **1995**, *11*, 1.
[15] R. Boraston, P.W. Thomson, S. Garland, J.R. Birch, *Devel. Biol. Stand.* **1984**, *55*, 103.
[16] J.G. Aunins, H.-J. Henzler, *Biotechnology* **1993**, *3*, 219.
[17] H.-P. Meyer, A. Fiechter, *Appl. Environm. Microbiol.* **1985**, *50*, 503.
[18] H.-P. Meyer, O. Käppeli, A. Fiechter, *Ann. Rev. Microbiol.* **1985**, *39*, 299.
[19] J. Varley, J. Birch, *Cytotechnology* **1999**, 29, 177.
[20] C. Leist, H.-P. Meyer, A. Fiechter, *J. Biotechnol.* **1990**, *15*, 1.
[21] U. Krebser, H.-P. Meyer, A. Fiechter, *J. Chem. Tech. Biotechnol.* **1988**, *43*, 107.
[22] H.-P. Meyer, in 'Physical Aspects of Bioreactor Performance', DECHEMA Frankfurt am Main, 1987, p. 144.
[23] F.W.J.M.M. Hoeks, *algroup lonza*, CH-3630 Visp (Switzerland).
[24] F.W.J.M.M. Hoeks, C. van Wees-Tangerman, K. Gasser, H.M. Mommers, S. Schmid, K. Ch.A.M. Luyben, *Canad. J. Chem. Engineering* **1997**, *75*, 1018.
[25] A. Jaquet, I.W. Marison, H.-P. Meyer, U. von Stockar, *Chimia* **1996**, *50*, 426.
[26] F.W.J.M.M. Hoeks, H. Kulla, H.-P. Meyer, *J. Biotechnol.* **1992**, *22*, 117.