Microbial Cell Factories

Poster Presentation

Potentials and limitations of prokaryotic and eukaryotic expression systems for recombinant protein production – a comparative view

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Background

Within the last recent years biopharmaceutical sales have reached 30% of all new pharmaceutical sales in the United States expecting an increase from 30 billion USD (2003) to almost 60 billion USD until 2010 [1]. One major industry of the fast growing biopharmaceutical market is the manufacture of recombinant proteins for therapeutic and diagnostic use. Hence, the rising demand for new biopharmaceuticals requires increased production capacities as well as new production processes that exhibit increased space-time yields and shortened development times which also implies the use of suitable expression systems.

In the 1970s, when recombinant DNA technology found its way into molecular biology laboratories, the bacterial cell was prevalently presented as a universal host for heterologous protein expression. However, due to their inability to adequately process complex proteins and due to their insufficient protein secretion capabilities prokaryotic expression systems nowadays are mainly used for the production of rather uncomplex proteins and peptides. In order to produce complex human recombinant proteins a reinforced development of eukaryotic expression systems has proceeded in the last decades, which was mainly based on yeast cells and mammalian cells. As a result, mammalian cell-based biopharmaceuticals account for almost 60% of today's biopharmaceutical market.

But, what do we really know about the production capacity of a particular host cell related to cell mass and cell volume? What can we learn in order to ease the choice of a suitable expression system for a particular protein? How can we use this knowledge to optimize the product yield related to process time and space?

We have evaluated different expression systems which have been technologically used for recombinant protein production: an inducible prokaryotic expression system (*Escherichia coli*) for intracellular human superoxide dismutase [2], an *E. coli* secretion system for antibody Fab fragments [3], a constitutive eukaryotic secretion system (*Pichia pastoris*) for human trypsinogen and antibody Fab fragments [4,5] as well as an intracellular *P. pastoris* system [6], and a constitutive eukaryotic expression system (CHO) for an EPO/Fc fusion protein and human monoclonal antibodies [7]. We developed production scenarios for each expression system and compared specific growth and productivity as well as product secretion rates to determine the full potential in a bioprocess.

Results

Based on own experience in our labs, as well as on literature data, we have developed three main scenarios for protein production, based on *E. coli*, *P. pastoris* and CHO cells as alternative host systems. Maximum and average values for specific growth rates, specific product formation (secretion) rates, and space time yields (volumetric productivities) are summarized in table 1.

Relating these data to total protein synthesis rates enables the estimation of the upper limits of production capacities, while the volumetric productivities inform about the economic efficiencies of the different scenarios.

Table 1: Comparison of typical max. cell dry masses, achieved product concentrations, specific product formation rates and volumetric productivities of the selected model processes.

	CHO cells	P. pastoris	P. pastoris	E. coli	E. coli
Destination of product	secreted	secreted	cytoplasm	secreted	cytoplasm
[$g \cdot L^{-1}$]	0.5 – 5	80 – 150	80 – 150	20	35
Typical spec. growth rate [h^{-1}]	0.02	0.02	0.02	0.1	0.1
Typical specific product formation rate [$mg \cdot g^{-1} \cdot h^{-1}$]	1.5 – 4.5	0.05 – 0.5	4	0.25 – 2.5	20
Product concentration (per culture volume) [$g \cdot L^{-1}$]	0.1 – 0.5	0.25 – 2.5	20	0.2 – 2	7
Volumetric productivity [$mg \cdot L^{-1} \cdot h^{-1}$]	1 – 2 (- 25 for perfusion)	1 – 6 (- 25 for continuous processes)	160	4 – 40	200

Conclusion

Up to now it was not usual practice to compare mammalian cell culture processes on a common basis with microbial processes. The presented data provide a clear basis to judge both the economic capabilities of present processes and the potential and constraints of the different host systems, pointing the attention to the most severe bottlenecks that limit the economic feasibility of the respective production systems. These data enable us to generalize the understanding of the biological limitations of protein synthesis and secretion, thus obviating the major potentials for the optimization of currently available expression systems.

References

1. Birch J: **Mammalian cell culture: current status, future prospects. Oral presentation.** *Cell Culture and Upstream Processing, Berlin* 2004.
2. Kramer W, Elmecker G, Weik R, Mattanovich D, Bayer K: **Kinetic studies for the optimization of recombinant protein formation.** *Ann NY Acad Sci* 1996, **782:**323-333.
3. Maier T: **An *E. coli* based secretion system for the production of proteins and Fabs.** *Bioprocess International, Prague* 2006.
4. Hohenblum H, Borth N, Mattanovich D: **Assessing viability and cell-associated product of recombinant protein producing *Pichia pastoris* with flow cytometry.** *J Biotechnol* 2003, **102:**281-290.
5. Gasser B, Maurer M, Gach J, Kunert R, Mattanovich D: **Engineering of *Pichia pastoris* for improved production of antibody fragments.** *Biotechnol Bioeng* 2006 in press.
6. Hasslacher M, Schall M, Hayn M, Bona R, Rumbold K, Luckl J, Griengl H, Kohlwein SD, Schwab H: **High-level intracellular expression of hydroxynitrile lyase from the tropical rubber tree *Hevea brasiliensis* in microbial hosts.** *Protein Expr Purif* 1997, **11:**61-71.
7. Trummer E, Fauland K, Seidinger S, Schriebl K, Lattenmayer C, Kunert R, Vorauer Uhl-K, Weik R, Borth N, Katinger H, Müller D: **Process parameter shifting: Part I. Effect of DOT, pH and temperature on the performance of Epo-Fc expressing CHO cells cultivated in controlled batch bioreactors.** 2006 in press.