Fed-batch based cultivation method improves yield of soluble recombinant proteins in shaken cultures

Application Note

Summary of published results [1], by Katharina Lehmann

Introduction

Cultivation for recombinant proteins is usually applied in batch mode. Here, hardly controllable conditions and low cell densities decrease the production rate of soluble proteins. Optimized media with favourable nutrients increasing cell growth, unfortunately might be toxic. Substrate-limited fed-batch cultivation, commonly used in bioreactors, provides a constant or intermittent release of a limiting substrate component to control growth rates, allowing cultivation in aerobic conditions. Feeding without external advises, as in the recently developed EnBase Flo system, uses a principle of enzymatic glucose release from a fully soluble polymer in liquid phase cultivation, which makes this cultivation method applicable and efficient for shaken cultures.

Examination of cell growth, pH development and protein yields could demonstrate how the novel EnBase Flo system provides a significant improve in production rates by increasing cell densities.

Method

In this cultivation three different strains of E. Coli were cultivated in EnBase Flo, conventional media: Luria Bertani (LB), Terrific Broth (TB) and mineral salt medium (MSM).

First, cultivation of all four media was obtained in normal round-bottom Erlenmeyer flasks and deep well plates (DWP24), starting at an OD_{600} of 0.15. In EnBase Flo (based on mineral salt media), the glucoamylase `EnZ I'm' was additionally added. During overnight cultivation, the growth in EnBase Flo could hereby be tightly controlled by enzymatic glucose feeding (glucose-limited fed-batch method).

Secondly, all cultures were run for 24 h with addition of IPTG inducer after two hours. Adding the growth booster together with IPTG provided efficient protein synthesis within a 3 to 6 hours period in EnBase Flo. The boosting solution benefits synthesis as complex nutrients (optimized combination of peptones, yeast extracts and trace elements) donate energy and help to maintain the required pH. A temporary and controlled increase in growth rate and a low product formation rate is achieved. The appearing growth rate close to starvation has shown to be optimal for correct folding in protein production, which makes unfavourable changes in temperatures and IPTG concentrations (0.4 or 1 mM) dispensable.
In the last period (16 to 21 h), the recombinant protein can be slowly synthesized and folded, due to low growth rates.

Cell growth was followed by optical density (OD_{600}) measurements with spectrophotometry. Total and soluble protein samples were analysed by SDS-PAGE. The bands were visualized by Coomassie Brilliant Blue staining. Prior to cell lysis the volume of Tris buffer per each sample was adjusted so that the resulting cell density was the same in all samples to compare protein productivity per cell in different samples.

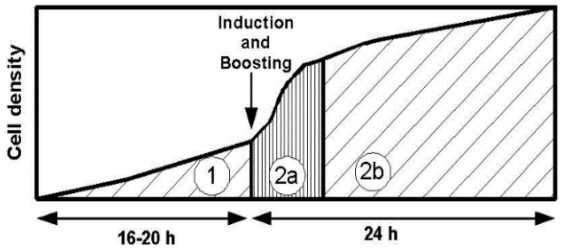

Figure 1: A schematic picture of the EnBase® Flo cultivation Phase 1: controlled cultivation in MSM with a low concentration of complex additives Phase 2: induction with IPTG and booster mixture. Phase 2a: increased growth rate Phase 2b: low growth rate and slow production of soluble protein. (Krause et al., 2010)

Results

Due to the growth control, accumulation of harmful metabolites can be minimized, and appearance of anaerobic conditions can be avoided. 10 to 20-fold higher induction cell density could be used. Hence, final cell densities were typically 10 times higher compared to the standard Sambrook protocol. The increase in cell density significantly improved the production of recombinant proteins.

In comparison to standard cultures in LB, Terrific Broth and mineral salt medium, typically over 10-fold higher volumetric yields of soluble recombinant proteins were achieved, since the productivity per cell of total and especially soluble protein was remarkably higher after a 24h induction period.
The yield of purified soluble protein significantly showed a higher amount of crystallization grade protein per volume in EnBase Flo.

A stable pH between 6.5 and 7.5 throughout the 43h cultivation could be maintained in each culture. The pH maintenance EnBase Flo is not regulated through a high concentration of buffer agents, rather than through the controlled delivery of glucose and nutrients, providing new possibilities for pH control and adjustability.

Strain	Cultivation format	Medium	OD_{600} at ind.	OD_{600} at harvest
RB791 [pQE30:adh]		LB	0.4	11.1
	24DWP	TB	0.6	12.7
		MSM	0.2	17.5
		Enbase Flo	9.3	51.3
BL21 (DE3) [pPal7:Dm_MFE-2]		LB	0.6	5.9
		TB	0.8	10.1
	24DWP	MSM	0.2	12.6
		Enbase Flo	12.1	31.7
BL21(DE3) pLysS [pET23:pdi]		LB	0.2	6.1
		TB	0.5	16.9
	24DWP	MSM	0.2	2.5
		Enbase Flo	12.1	33.3
Strain	Cultivation format	Medium	OD_{600} at ind.	OD_{600} at harvest
RB791 [pQE30:adh]		LB	0.9	8.8
	shake flask	TB	0.7	17.1
		MSM	0.3	9.8
		Enbase Flo	9.2	32.2
BL21(DE3) [pPal7:Dm_MFE-2]		LB	0.7	4.4
	shake flask	Enbase Flo	12.8	24.7

OD_{600} and pH at induction and at final cell harvest (24 h

Table 1:
Cell growth in all cultivations at induction and harvest (Krause et al., 2010)

The EnBase Flo cultivation method offers a good scalability since it is not bound to a certain promoter system and can be scaled-up from 3 ml to 50 ml culture volume in 500 ml shake flask without deterioration of the protein production. Several cultivations can be performed at the same time by simply decreasing cultivation volume, enabling further use of microwell or deep well plates, applicable in robot-driven automated cultivations. Although the whole cultivation takes more time to perform than in standard system, it is easier and faster to perform. Additional fine-tuning can be easily achieved by enzyme dosing. EnBase Flo is useful in screening and automatic sample treatment, or implementation in high throughput screening purposes in small scale, due to higher cell densities.

Conclusion

Compared to commonly used shake flask cultivations, applying EnBase Flo cultivation system in shaken cultures can obtain higher cell densities, higher productivity per cell and a more adequate pH control. Thus, the yield of soluble proteins improves significantly without impairing cell productivity. The improvement is thought to result from the well-controlled fed-batch process, based on enzymatic glucose release in a well-balanced medium. It is widely and easily applicable and can be used in various biotechnological processes.

Reference

Krause, Kaisa Ukkonen, Tatu Haataja, Maria Ruottinen, Tuomo Glumoff, Antje Neubauer, Peter Neubauer, Antti Vasala. Microbial Cell Factories 2010, 9:11

http://www.microbialcellfactories.com/content/9/1/11