

# Maximising the process yield for a recombinant fusion protein by fermentation regime optimisation

Christian Kaiser, Stefan Krüß, Michael Küchler\*  
Richter-Helm BioLogics GmbH & Co. KG, Hamburg, Germany

\* Contact to: [info@richter-helm.eu](mailto:info@richter-helm.eu)

## Introduction

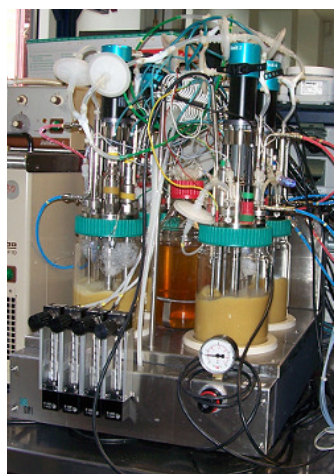


Fig. 1: Fermentation Equipment

### Trends in Microbial Fermentation

For the development of upstream processes in the biopharmaceutical industry, achieving high product yields in combination with process robustness, a systematic screening of key parameters (Tab. 1) is a prerequisite. Systematic parallel approaches in multi-fermenter systems keep the time, materials and effort to a minimum while at the same time providing a better process understanding.

Applying the principals of DoE during USP development leads to the identification of critical quality attributes (CQA) and thus to a better process understanding. CQA characterisation is in accordance to the QbD approach of ICH Q8 providing first relevant information for process validation.

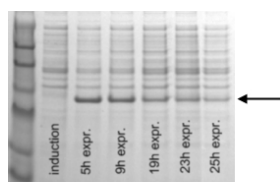
### Project Situation

In the given example a recombinant therapeutic fusion protein is expressed primarily in inclusion bodies. The *E. coli* BL21 host strain is transformed with a heat inducible expression plasmid constructed by Richter-Helm BioLogics.

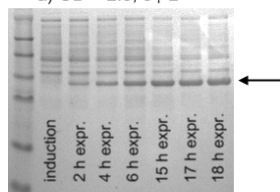
Parameter	Variation
Medium <sup>1,2</sup>	mineral, semi defined or complex
	trace elements
	amino acids
	vitamins
Feeding <sup>1,5</sup>	linear, exponential or combined profiles
Induction <sup>2,3,4,5</sup>	induction temperature, timeframe for induction
	inducer concentration, inducer addition
pH <sup>4,5</sup>	reference value
	pH profile
Temperature <sup>2,4</sup>	reference value
	temperature profile
pO <sub>2</sub> <sup>5</sup>	reference value

Tab. 1: Key parameters

## Results



a) OD = 2.5, 5 µL



b) OD = 2.5, 5 µL

Cultivations were performed in fed-batch mode on a semi-defined media. In a first attempt a strong target protein formation was observable during the first four to six hours after induction (Fig. 2a). Thus a fusion protein titre of ~1.2 g/L was determined.

Applying an exponential feeding profile and the addition of vegetable peptone in the feed media led to a slight increase in target protein yield (~1.5 g/L). However, the time interval of strong protein expression was not prolonged.

In order to reduce the cell stress caused by the high induction temperature and strong expression rate, different temperatures for induction were investigated in the following step. As visible in Fig. 2b and Fig. 3 strong protein expression is sustained for the overall expression phase at an induction temperature of  $\vartheta_{L,ind} = 38^\circ\text{C}$ . Subsequent optimisation by induction time point shift and scale-up led to a product titre of ~24 g/L.

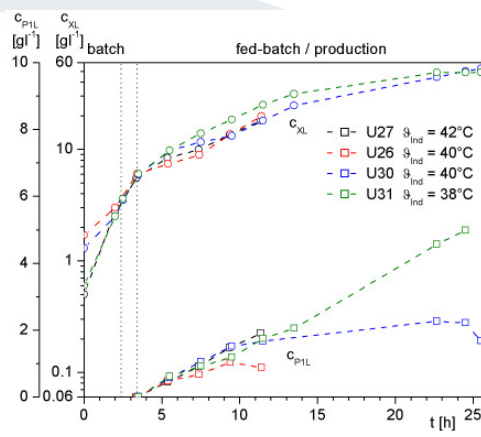


Fig. 3: Fermentation trends

## Discussion

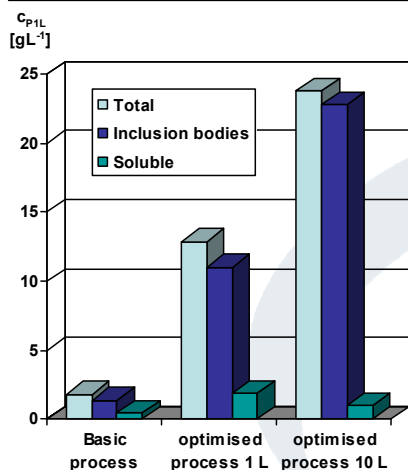


Fig. 4: Yield improvement

During the development phase for the upstream process initial fusion protein yield was increased 10 times in a 1 L multi-fermenter system. Process upscale to a 10 L system led to a further increase in the target protein titre due to better mass transfer rates and thus an extended expression phase and product yield (Fig. 4).

Increased productivity leads to lower cost of goods (COG), decreased facility time and thus increased flexibility for the production unit.

Applying a systematic parameter screening during USP development and robustness testing by means of DoE leads to a first characterisation of the fermentation process and enables the definition of target values in a defined design space for subsequent GMP-runs. Exemplarily a contour plot for the dependency of the target protein formation on feeding rate and pH is displayed in Fig. 5.

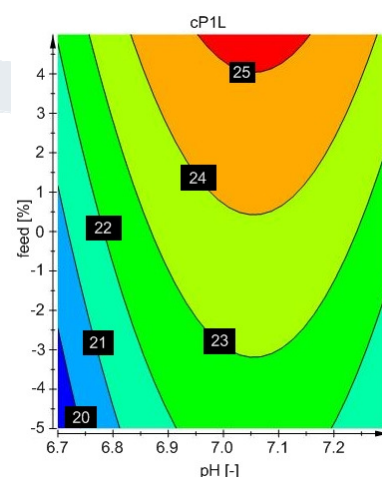


Fig. 5: Expression dependency

<sup>1</sup> Eiteman, M.A. and Altman, E. (2006) *Trends Biotechnol.*, **24** (11), 530-536

<sup>2</sup> Sorensen, H. and Mortensen, K. (2005) *Microb. Cell Fact.*, **4** (1), 1

<sup>3</sup> Ramirez, D.M. and Bentley, W.E. (1995) *Biotechnol. Bioeng.*, **47** (5), 596-608

<sup>4</sup> Strandberg, L. and Enfors, S.-O. (1991) *Appl. Environ. Microbiol.*, **57** (6), 1669-1674

<sup>5</sup> Choi, J.H., Keum, K.C. and Lee, S.Y. (2006) *Chem. Eng. Sci.*, **61** (3), 876-885