

Screening and Fermentation Process Development for the Yeast *Hansenula polymorpha* Using 16-fold Parallel Fed-batch Fermentation

Background

The DASGIP fed-batch pro device (DASGIP AG, Jülich, Germany), a 16-fold parallel fed-batch bioreactor, was used for the high-cell-density fed-batch cultivation of *Hansenula polymorpha*. This yeast is established as an expression host for recombinant proteins and is being used on an industrial scale, e. g. for the production of recombinant hirudin.

The fermentation strategy for *Hansenula polymorpha* employs a growth phase in which the cells are fed with a fixed-ratio mixture of glycerol and ammonia at a feed rate set by the pH controller. At high cell densities, the fermentation is switched to the so-called „derepression phase“, in which 50 % glycerol is fed at limiting rates regulated by the dissolved oxygen controller while pH is controlled independently using 25 % ammonia solution.

The DASGIP fed-batch pro device available at Fraunhofer IME/RWTH Aachen features 16 vessels of 200-275 ml working volume. Temperature can be controlled independently for groups of four vessels. Aeration can be carried out as headspace aeration or via ring spargers. Agitation is provided by magnetic stirrers whose speed can be controlled independently for groups of four vessels. Each vessel is connected to three feed lines whose feed rate can be manually adjusted or programmed.

Project aim

Our industrial partner's problem was to screen a number of *H. polymorpha* expresser clones producing a protein of therapeutic relevance. The eight expresser clones had been identified in shake-flask based expression trials as good producers. However, these findings needed to be verified in a stirred-tank-

reactor system and using a high-cell-density fed-batch fermentation strategy, because results from shake-flask experiments cannot always be transferred to the bioreactor level. The results obtained in the DASGIP fed-batch pro screening were to be verified in 5-L stirred-tank reactors.

Experimental procedure and results

Growth in the 16 vessels of the DASGIP fed-batch pro each containing 260 mL of minimal medium was monitored by measuring OD₆₀₀. Fig. 2 shows the growth fermentation diagram for one of the 16 vessels. As the cultures reached an OD₆₀₀ of 125 to 150 after 43 to 50 hours, they were individually shifted to the derepression phase. During this phase, glycerol was fed at a growth-limiting rate. The experiment was terminated after 91 h, with OD₆₀₀ values between 221 and 425. These OD values correspond to a biomass fresh weight of 160 to 298 g·l⁻¹. Samples were analyzed for product content by RP-HPLC (Figure 3).

The two clones identified as best expressers in the DASGIP fed-batch pro experiment were run in a stirred-tank reactor fermentation at the 5-L scale (data not shown). The fermentation and production characteristics observed in the small-scale screening could be verified in these experiments (data not shown). Product concentration in the harvest supernatant reached levels of up to 1.2 g/L as determined by RP-HPLC.

Conclusions

In only one week, eight different expresser clones of *Hansenula polymorpha* were screened in duplicate experiments in a parallel fed-batch fermentation device. Following a rugged fermentation strategy, fermentation conditions close to those in industrial processes were applied. The fermentation and production characteristics could be verified in 5-L scale bioreactors, thus demonstrating the potential of parallel fermentation technology to improve the „time-to-market“ for recombinant proteins.

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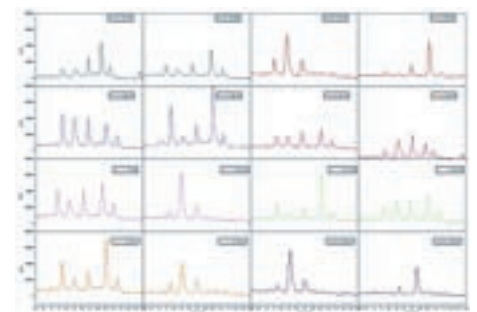


Figure 3:
RP-HPLC of samples taken from the 16 vessels.
Identical colours reflect duplicate experiments.