Optimization of a Fed-batch Strategy for Production of Mab Ign 311 in Small Scale Experiments

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Abstract: We demonstrate here an optimization of a feeding strategy in standard grade roller bottles in a 400 mL scale. Knowledge of critcal parameters, e.g. nutrients shortage, was incorporated in our feeding strategy and thereby final product concentration could be increased by 75% compared to non-feeded standard cultures. Combined with optimized media increases in product titers of about 125% could be achieved. These optimization steps had no negative impact on product characteristics. At the end, a strategy ready for further experiments in bench-top bioreactors could be presented.

Key words: Feeding strategy, fed batch, small scale, roller bottles, bench-top bioreactor, consumption rates, SP2/0, IGN311, hydrolysate, Hypep 4601, product quality

1. INTRODUCTION

Igeneon is currently testing a humanized monoclonal antibody (IGN311) specific for Lewis Y, a carbohydrate antigen, in clinical trials of passive cancer immunotherapy. A Phase I trial was just finished, and a Phase II study has to be supported with sufficient material. To meet this increasing demand the production process has to be adjusted.

As not all experiments can be performed in large scale, we tested a variety of process parameters, e.g. the feeding strategy, in roller bottles.

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2. MATERIAL AND METHODS OF ROLLER BOTTLE EXPERIMENTS

Production cell line for this antibody is a murine Sp2/0. Tests were performed in 850 cm² roller bottles (Corning, Germany) with a starting volume of 400 mL. Inoculation density was 1.5 x 10^6 cells mL^{-1}. Experiments were performed at 37 °C in an incubator with 7% CO2.

Antibody concentration of samples was analyzed by SEC-HPLC. Standard was IGN311 Batch 213601 (Igeneon, Austria). This material was produced in a hollow fiber process using a medium which was supplemented with 10% FCS. Feeding media was a concentrate of our cultivation media, glutamine was supplemented separately.

3. DATA OF ROLLER BOTTLE EXPERIMENTS

First experiments were aimed to describe the effect of adding L-glutamine at a later stage in cultivation. As expected feeding prolonged cultivation time and cell densities (2.0 x 10^6 cells mL^{-1} on average in fed batches versus 1.6 x 10^6 cells mL^{-1} for the batch). Product titers were increased by later addition of L-glutamine by 20%, and by the use of Hypep (HP) 4601 in the feeding media by 30%. These results were very promising for the further design of our fed batch strategy, and in our next experiments we combined these two effects.

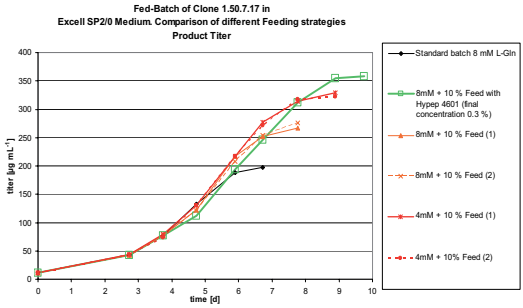

Figure 1. Cultures containing 4mM L-Glutamine were fed on day 4 and 4mM L-Gln was added on day 6. Cultures containing 8 mM L-Gln were fed on day 6. Longer cultivation time reflects in higher product titers as can be seen in this graph.

In the next experiment positive effects could be verified, titers could be increased about 15% just by supplementation of HP 4601 to the feeding media, and up to 35% by using the hydrolysate as component of the

cultivation media. As a consequence it was decided that HP 4601 will be taken as standard supplement for further fed batch experiments.

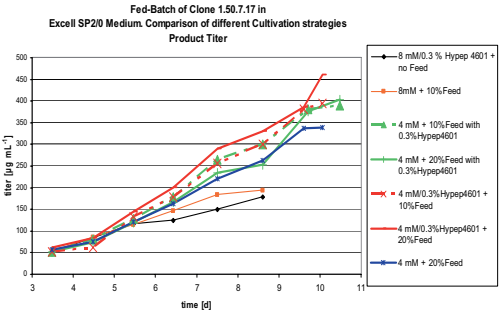

Figure 2. Combination of addition of HP 4601 and delayed supplementation of L-Glutamine (was generally added to 4mM -cultures on day 6). Longer cultivation time and higher maximum cell number accumulates in final titers.

4. QUALITY TESTING OF IGN311

Analyses of the binding affinity by anti-idiotypic ELISA and anti-Lewis Y ELISA revealed no changes in affinity between IGN311 standard and IGN311 produced with up to 0.5% Hypep 4601. When compared to IGN311 standard, material derived from cultures containing Hypep 4601 (batch and fed-batch) displayed ADCC activities generally 2 to 4 times higher, CDC activity was elevated between 10% and 30%. The IEF pattern was not changed by whatever kind of feeding strategy or supplementation. Compared to IGN 311 standard, all samples own two additional band in the alkaline region (pI 8.4 – 8.1). This is mainly caused by the switch from FCS containing production media (for standard) to serum free media.

5. CONSUMPTION AND PRODUCTIVITY RATES AND BIOREACTOR CULTURE

Specific productivities stayed constant throughout cultivation in all roller bottle experiments and were in the usual range of variation of standard cultures (40 ± 4 pg cell^{-1} d^{-1}).

We calculated average consumption rates based on cumulative cell-days of cultures in basal media with 8 mM L-Gln. Afterwards we compared them with consumption rates in the early stage of a discontinuous fed-batch

containing 0.5% HP 4601 in its media. This fed batch was performed in a small scale bioreactor (Minifors 2.5L, Infors, Switzerland).

In this bioreactor culture consumption rates of almost all amino acids were reduced. This effect is caused by the addition of HP 4601, as it presents a resource of various peptides which can be utilized by our host cells. Critical amino acids have to be considered in respect of feeding, as their content decreased during cultivation in the fed- batch up to 50%.

Glutamine is the mayor energy resource of our IGN311 clone. We found that its consumption is increased during bioreactor cultivation in HP 4601 containing media by about 60%. Glucose consumption stayed almost unchanged in the bioreactor (2.2 ± 0.1 µmol*10^{-6}cells*day^{-1}) compared to average values for roller cultures without HP 4601 (2.4 ± 0.1 µmol*10^{-6} cells*day^{-1}]. Although feeding rate in this bioreactor fed-batch was not fully optimized, maximum viable cell number of 5×10^6 cells mL^{-1}, and a final product titer of about 500 µg mL^{-1} could be achieved.

6. CONCLUSIONS

- Roller bottle experiments were able to support assessment of feeding strategies and verification of selection of suitable media additive (Hypep 4601).
- In roller bottles increases in product titers of almost 125% were achieved, maximum cell number could be increased up to 25%. Cultivation time could be prolonged by 2 to3 days.
- Material derived either from batch or fed-batch cultures showed slightly enhanced lytic capacity compared to standard produced in serum-containing media.
- Critical amino acids (Asn, Leu, Lys) could be identified and an increase in Gln-consumption could be observed in a small-scale bioreactor cultivation with discontinuous feed.