**Aptitude of a microbioreactor as high throughput screening platform for cultivation process development**

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**Highlights**

* Microbioreactor enables high throughput clone screening
* Microbioreactor enables fermentation process parameter screening
* Fab expression causes lysis after a specific timespan

**1. Introduction**

We selected Antigen binding fragments (Fabs) as model proteins to evaluate the potential of a microbioreactor system. For this class of molecules a broad range of possible combinations of genetic constructs (host, leader sequence, promoters, mono- or bicistronic constructs, order of heavy and light chain sequences, UTR sequence etc.) is intensively discussed and consequently Fabs are optimally suited to answer this question. Fabs are single monovalent binding arms of IgG molecules, composed of a light and a heavy chain connected by disulphide bonds. As they are distinctive smaller in molecular size than full antibodies, they get producible in *E. coli* which makes it an attractive alternative to mammalian cell culture [[1-3](#_ENREF_1)].

In the course of this work the main focus was laid on the applicability of a microbioreactor for clone and condition screening and transferability of results to bench top fed batch fermentation processes. The main challenge is to screen a high amount of clones in the shortest possible time, getting significant and reliable data.

**2. Methods**

We selected four different Fabs (BIBH1, BIWA4, CIM and FabX) with identical constant domains. For translocation to the periplasm a post-translational (ompA) and a co-translational (dsbA) leader sequence were used. *E. coli* BL21(DE3) and *E. coli* HMS174(DE3) were transformed via genome integration resulting in a total number of 16 clones.

For a first screening of the expression systems a microtiter fed batch like cultivation in a BioLector® system was used. After a growth phase of 16 hours we induced the systems with 0.5 mM IPTG and after a 9 hours production phase samples for the determination of intracellular Fab concentration by ELISA were taken.

In a next step we performed fed batch cultivations with all clones in a DASGIP® cultivation system. After a batch phase at 37 °C with a final biomass of approximately 10 gCDM/L a C-limited feed phase was started with a growth rate of 0.1 h-1 at 30 °C. After approximately 0.5 doublings recombinant protein expression was induced for another two doublings. Induction level was kept constant at 2 µmol IPTG/g CDM.

**3. Results and discussion**

During BioLector ® cultivation Cell growth was not affected by leader/Fab combinations but yield of correctly folded Fab ranged from 0 to 12.5 mg/gCDM. In general K12 clones showed higher specific Fab titers but lower biomass yields than BL21 clones. The microbioreactor fed batch like cultivations with the selected set of host/leader/Fab combinations resulted in a broad range of variations in terms of Fab yields.

During bench top experiments we end up with exactly the same clone ranking in terms of specific Fab yield. However we also observed lower specific yields in the benchtop bioreactor setup which can mainly be assigned to the significantly higher growth rate in the production phase. In addition the production period was longer and approximately 8 hours past induction adverse effects on cell growth were observed. Fab was not only found in the cell fraction but also in the supernatant and DNA quantification in the fermentation supernatant showed that Fab release was caused by lysis which is also highly negatively correlated with the final cell mass that was obtained. The most significant influence on growth was seen for Fabx clones if the Fab was expressed.

**4. Conclusions**

Summarizing we could show that the microbioreactor system is a well suited HTP tool for clone and condition screening. Results generated in this platform can directly be transferred to benchtop fed batch bioreactor cultivations as long as limitations of the HTP system are adequately included in interpretations.

**References**

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