Method establishment for determination of protein expression related parameters

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Introduction

The scientific understanding of bioprocesses is of utmost importance for life sciences industries in order to cope with changes in registered pharmaceutical processes. These changes may result from unwanted deviations of the running process, as well as from intended strain improvements after the process has been registered. In order to gather scientific knowledge in a comprehensive but also effective way, innovative tools along process analytical technology (PAT) and new strategies for quality by design (QbD) have to be developed. The goal of this work is to establish a methodology to characterize a recombinant protein production process with the help of different cultivation methods like batch and fed-batch.

Experimental Design

Table 1. Comparison of yields and productivities

<table>
<thead>
<tr>
<th>Specific productivity [U/g]</th>
<th>Medium</th>
<th>Yield [mmol/C-mol]</th>
<th>Productivity [U]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed-batch</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Batch</td>
<td>0.58</td>
<td>1.00</td>
<td>0.58</td>
</tr>
</tbody>
</table>

HPR activity was detected both in the absence and in the presence of D-Ala, with slightly better results in the latter case, therefore D-Ala was added to the fed-batch cultures.

Results

Comparison of yields and productivities

- Only specific data (as specific rates and yields) showed to be useful to compare two fermentation modes as batch and fed-batch.
- We determined higher specific growth rates and specific uptake rates in pulsed batch cultures than for continuous limited fed-batch fermentations. The performance of the methanol fed-batch was suboptimal when compared to the output of the induction by pulses in batch. This can be explained by the lower specific growth rate at which the fed-batches were controlled. Specific productivity was almost the same in both cultivation system.

Conclusions

- The yields of CO₂ on methanol were lower for batch cultivation, which leads to the assumption that the cells in fed-batch cultures produced more CO₂ for metabolic maintenance and not for cell growth.
- On the other hand, the yield of product on methanol was significantly higher in fed-batch cultures. There is more potential for production of protein using controlled fed-batch regimes. Adjusting feeds for higher specific uptake rates could lead to improved protein production without loss of energy in growth and maintenance metabolism.
- Outlook – optimized feed by using online measured Yields (e.g. YCOD/MeOH) for q₁ control to increase volumetric productivity.